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NOUVELLES RECHERCHES SUR LA FACILITATION ET L'INHIBITION DES POTENTIELS ÉVOQUÉS CORTICAUX DANS L'ÉVEIL RÉTICULAIRE

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INTRODUCTION

Les recherches qui font l'objet du présent travail ont eu comme but de préciser le mécanisme des modifications des potentiels évoqués corticaux dans l'éveil cérébral provoqué par une stimulation électrique des formations réticulaires mésencéphalique ou thalamique ou par une excitation sensorielle, et de dégager, si possible, la signification fonctionnelle de ces modifications.

MÉTHODES

Les expériences ont été, pour la plupart, exécutées sur la préparation *encéphale isolé* du chat. Quelques unes ont été faites sur l'animal (chat ou lapin) immobilisé au flaxédil. Les techniques de stimulation et de dérivation des potentiels évoqués ont été décrites dans deux mémoires précédents (4, 5). Nous rappelons que nos expériences ont comporté la stimulation bipolaire du nerf optique (après évidement du globe oculaire), du corps genouillé latéral ou médian, de la formation réticulaire mésencéphalique paramédiane. Les impulsions électriques rectangulaires, isolées ou couplées ou répétitives, avaient en général une durée de 0,3 msec. Les électrodes de stimulation centrale, de même que les électrodes de dérivation souscorticale, étaient insérées stéréotaxiquement selon les coordonnées de l'atlas de Jasper et Ajmone-Marsan (22). Elles étaient, les unes et les autres, soit du type bipolaire simple (fils d'acier, distance inter-électrodique de 1 à 2 mm), soit du type concentrique bipolaire (fils de cuivre de 0,1 mm de diamètre, distant de 0,4 mm au niveau de leurs pointes dénudés sur une longueur de 1 mm). Dérivation monopolaire des potentiels corticaux par électrodes humides montées sur spires d'argent chloruré. Enregistrement par tubes cathodiques à double canon, après amplification différentielle à liaisons capacitaires (constante de temps habituelle de 0,1 sec) et réduction des artefacts de stimulation par un dispositif de radiocouplage.

RÉSULTATS

1. *Interaction de la facilitation réticulaire des potentiels évoqués avec d'autres processus de dynamogenèse corticale.* — On sait que pour observer la facilitation réticulaire il est nécessaire d'appliquer le stimulus provoquant la réponse de l'aire de projection étudiée sur le noyau de relais thalamique correspondant à cette aire ou bien encore, dans le cas de l'aire visuelle, sur le nerf optique ou sur le chiasma (11, 3, 4, 5, 30, 31). Cette position des électrodes stimulantes a l'avantage de permettre d'observer parallèlement les modifications fonctionnelles de la réponse de l'écorce visuelle et du noyau de relais thalamique à la volée d'influx corticipètes.

Rappelons que la dynamogenèse du potentiel évoqué cortical comporte deux éléments distincts: *a*) une augmentation, qui peut être considérable, de son amplitude de sommet à sommet; *b*) une accélération de la succession de ses deux phases positive et négative dans les enregistrements en dérivation monopolaire de surface.

La potentiation, qui est d'égale importance pour ces deux composantes, est sans doute essentiellement l'expression de l'augmentation numérique, par un processus de recrutement, des neurones participant à la réponse. L'amplification de la phase surface-positive de celle-ci s'observe encore après l'abolition, à la suite d'une narcose corticale locale, de la phase surface-négative de la réponse, présumée d'origine dendritique, fait qui doit inciter à la prudence en ce qui concerne la tendance actuelle (cf. 35) à rapporter les effets des influx réticulaires sur le néocortex à la modification fonctionnelle qu'ils exerceraient électivement sur l'appareil dendritique de l'écorce. Par ailleurs, l'accélération caractéristique de la succession des deux phases de la réponse est une claire indication de la facilitation des transmissions synaptiques interneuroniques ascendantes dans l'écorce.

Le recrutement neuronique qui s'exprime ainsi par l'augmentation de voltage de la réponse statistique que représente le potentiel évoqué doit nécessairement puiser dans le contigent de neurones excités sous-liminairement par le stimulus testant appliqué seul. Il était intéressant de vérifier si cette frange sous-liminaire de la réponse (Sherrington) se révèlerait être commune à la facilitation réticulaire d'éveil et à d'autres mécanismes de facilitation du potentiel évoqué cortical. C'est effectivement ce qui a été observé, dans le cas de l'aire visuelle, pour les facilitations par illumination rétinienne selon Chang (6) et par sommation temporelle de deux volées d'influx afférents.

La Fig. 1 montre tout d'abord en *A* et *B* la potentiation de la réponse de l'aire visuelle qui résulte de la sommation temporelle de deux volées égales d'influx géniculo-corticaux, se succédant à l'intervalle de 12 msec. En *C* et *D*, une dynamogenèse de la réaction corticale a été réalisée par une brève stimulation réticulaire (200/sec, 2 V) précédant immédiatement l'application du stimulus testant. On voit que cette potentiation est d'importance égale à celle que réalisait en *A* (réponse seconde) la facilitation temporelle, ce qui signifie que le recrutement neuronique dans les deux facilitations est quantitativement très comparable. Si maintenant on fait succéder à la réponse facilitée par la stimulation réticulaire une réponse seconde, au même intervalle des deux stimuli testants que celui qui donnait en *A* l'effet de sommation temporelle, celui-ci est remplacé par une dépression de la réponse seconde conditionnée (Fig. 1, *C* et *D*). Tout se passe donc comme si la facilitation réticulaire et la facilitation

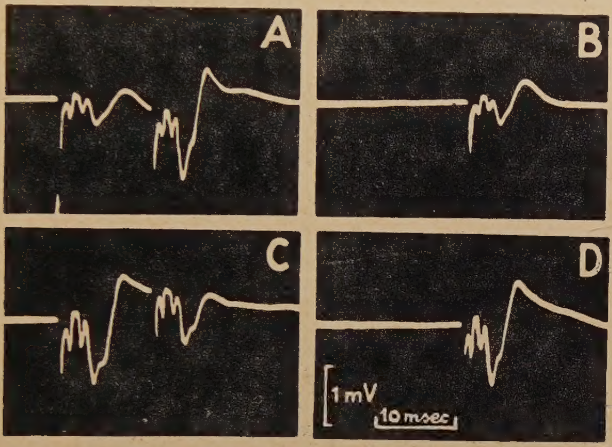

Fig. 1. – *Absence d'additivité des facilitations corticales par sommation temporelle et par stimulation réticulaire.*

Chat, encéphale isolé. Potentiels évoqués de l'aire visuelle en réponse à la stimulation du corps genouillé latéral.

A: facilitation par la succession de deux chocs genouillés identiques. *B*: réponse au choc second non conditionné. *C*: succession des deux mêmes stimuli qu'en *A*, mais précédés d'une brève stimulation de la formation réticulée mésencéphalique (200/sec; 2 V; 0,3 msec). *D*: réponse au choc second seul conditionné par la même facilitation réticulaire qu'en *C*. Autres explications dans le texte.

par sommation temporelle recrutaient, dans la frange sous-liminaire de la réponse au stimulus de contrôle, approximativement le même contingent de neurones disponibles.

Une constatation semblable a été faite dans des expériences où était étudiée, sur l'aire visuelle, l'effet additif d'une facilitation réticulaire et d'une facilitation par illumination rétinienne de la réponse à un choc genouillé testant.

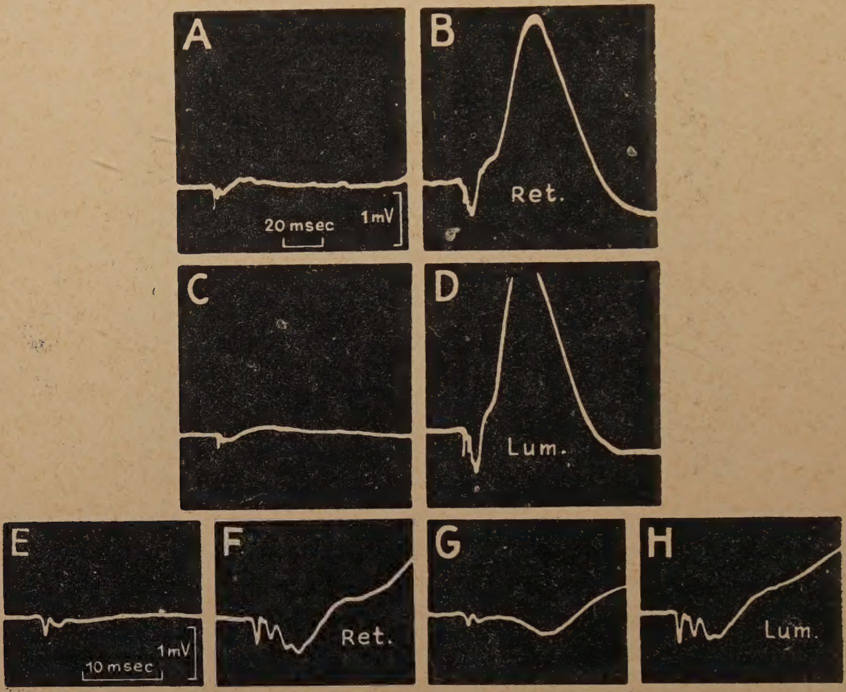

Fig. 2. – *Intensité égale des facilitations réticulaire et photique de la réponse de l'aire visuelle strychnisée à un choc « genouillé ».*

Chat, encéphale isolé. L'application d'une solution de sulfate de strychnine à 2,5°/₀₀ pendant une minute sur l'aire visuelle n'a pas modifié le potentiel évoqué de celle-ci (*A*, *C*, *E*, *G*). Mais l'éveil cortical provoqué par une brève stimulation de la formation réticulaire mésencéphalique précédant immédiatement le choc genouillé (*B* et *F*) ainsi que l'illuminiation rétinienne (*D* et *H*) révèlent toutes deux la strychnisation latente de l'écorce par l'augmentation énorme et approximativement égale du potentiel évoqué qu'elles provoquent. Noter la différence des échelles de temps en *A*-*D* et *E*-*H*.

Dans l'expérience illustrée par les tracés de la figure 2, les conditions des observations sont plus complexes parce que l'aire visuelle avait subi une application préalable de sulfate de strychnine à la concentration de 2,5°/₀₀. Cette strychnisation locale n'avait eu aucun effet apparent sur la réponse corticale au stimulus genouillé lorsque celle-ci était provoquée dans l'obscurité (Fig. 2, *A*, *C*, *E*, *G*). Mais l'effet strychnique apparaissait régulièrement lorsque le même stimulus testant était immédiatement précédé d'une brève stimulation de la formation réticulaire mésencéphalique (*B*) ou bien lorsqu' il était appliqué pendant l'illumination de la rétine (*D*). Les tracés *B*, *D*, *F*, *H*, enregistrés à des vitesses de balayage différentes (beaucoup plus rapide en *F* et *H* qu'en *B* et *D*), montrent la complète similitude des potentiations produite par les deux mécanismes différents de facilitation. Cette similitude se marque non seulement par le voltage à peu près égal de la composante surface-négative de la réponse en *B* et *D*, mais encore (*E* à *H*) par l'amplification très comparable des pointes (*spikes*) initiales de la réponse. On sait qu'en ce qui concerne la pointe 1 (potentiel d'action des radiations optiques), cette amplification est l'expression de la facilitation des transmissions synaptiques dans le corps genouillé latéral (11, 3, 4). Le

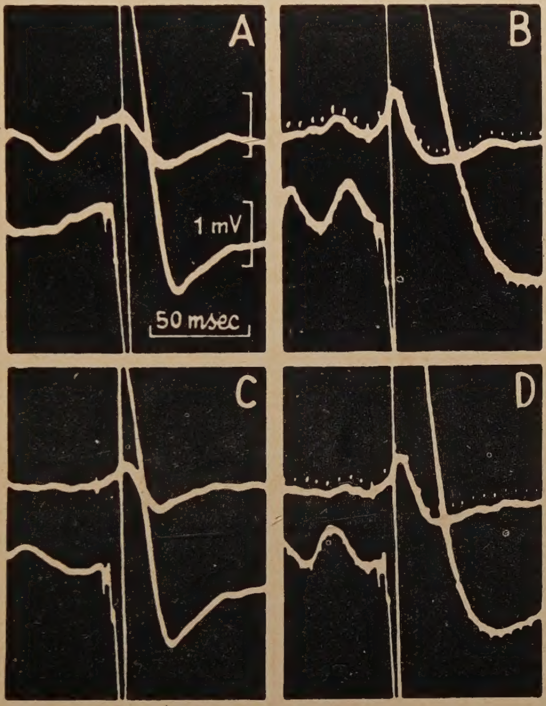

Fig. 3. – *Additivité des facilitations réticulaire et strychnique.*

Chat, encéphale isolé. Dérivation monopolaire des aires auditive primaires droite (tracés inférieurs de chaque paire) et gauche. L'aire auditive droite, a été strychnisée localement, plusieurs fois et à de longs intervalles, avec une solution 2,5°/₀₀. La réponse au clic est fortement amplifiée dans ses phases positive et négative (*A* et *C*). En *B* et *D* la stimulation de la formation réticulaire mésencéphalique (200/sec; 3,5 V) renforce encore considérablement le potentiel évoqué de l'aire auditive droite, et aussi (par transfert callocal) celui du point symétrique de l'aire gauche. Tracés reproduits dans l'ordre de leur enregistrement.

recrutement neuronique à l'intérieur du noyau de relais, que ne pouvait évidemment pas avoir affecté la strychnisation locale de l'écorce, était donc lui aussi d'importance pratiquement égale dans les deux types de facilitations, réticulaire et par illumination.

L'existence d'un plafond pour la dynamogenèse, que révèlent des expériences comme celle de la figure 1, ne se vérifie plus par contre lorsque l'on superpose une facilitation réticulaire à une potentiation strychnique *manifeste*. L'examen des tracés de la figure 3 va préciser cette notion. Dans cette expérience, la réponse de l'aire auditive primaire à un stimulus appliqué sur le corps genouillé médian avait été considérablement amplifiée dans ses deux phases à la suite d'une application locale de strychnine sur le point cortical exploré (tracés *A* et *C*). Cette dynamogenèse était telle qu'il semblait que le recrutement des neurones disponibles dans la frange sous-liminaire de la réponse fût maximum. Il n'en était rien cependant car la facilitation réticulaire eut régulièrement comme effet d'augmenter encore considérablement l'amplitude des deux phases, positive et négative, de la réponse au choc genouillé (tracés *B* et *D*). Plus tard lorsque l'effet strychnique manifeste avait disparu, la facilitation réticulaire révélait, comme dans les tracés *B* et *D* de la figure 2, la modification fonctionnelle latente de l'aire corticale intoxiquée.

Tout se passe comme si l'intoxication strychnique de l'écorce avait comme effet à la fois le recrutement d'un nombre plus ou moins grand de neurones de la frange sous-liminaire et un élargissement considérable de cette frange par l'entrée dans celle-ci d'éléments n'en faisant pas normalement partie (cf. 14). Par contre, les facilitations réticulaire et photique ne feraient que révéler l'existence de la frange sousliminaire sans en déterminer l'élargissement.

2. *Interprétation de l'inhibition* (« *masquage* ») *des potentiels évoqués dans l'éveil cortical.* — Cette dépression est comme on sait le résultat habituel de l'éveil par stimulation réticulaire et de l'éveil sensoriel lorsque le potentiel est provoqué par une brève stimulation des récepteurs périphériques. Cette donnée expérimentale concernant l'endroit d'application du stimulus sur la voie afférente étudiée suggérait la possibilité que la réduction de la réponse corticale provoquée par un stimulus « périphérique » fût la conséquence d'un processus d'inhibition active bloquant les influx sensoriels au niveau de l'un de leurs relais sous-corticaux infrathalamiques. L'existence

de semblables blocages a été mise en évidence dans l'attention, la distraction et l'habituation (15, 17, 18, 19, 20, 23). Une activation réticulaire a été incriminée en ce qui concerne le mécanisme opérationnel de ces blocages (20), non sans de sérieuses objections, basées sur des données expérimentales et des contrôles anatomiques précis (10).

Cette explication du phénomène de masquage par un blocage sous-cortical de l'afférence corticipète s'appuie sur le fait qu'une certaine réduction de voltage de la réponse des corps genouillés latéral ou médian peut effectivement accompagner, dans l'éveil réticulaire, l'affaiblissement du potentiel évoqué cortical. Il ne semble pas cependant que ce blocage constitue un facteur important de l'affaiblissement de la réponse corticale. Et cela pour les raisons suivantes. La réduction de la réponse du noyau de relais thalamique est dans l'ensemble beaucoup moins marquée que celle du potentiel évoqué cortical, qu'il s'agisse de l'aire visuelle (18, 4), de l'aire somesthésique ou de l'aire auditive (9). Elle peut même être nulle. Le même fait a été observé sur le lapin non narcotisé. Dans cinq expériences de stimulation photique la réduction d'amplitude très importante de la réponse de l'aire visuelle dans l'éveil électroencépha-

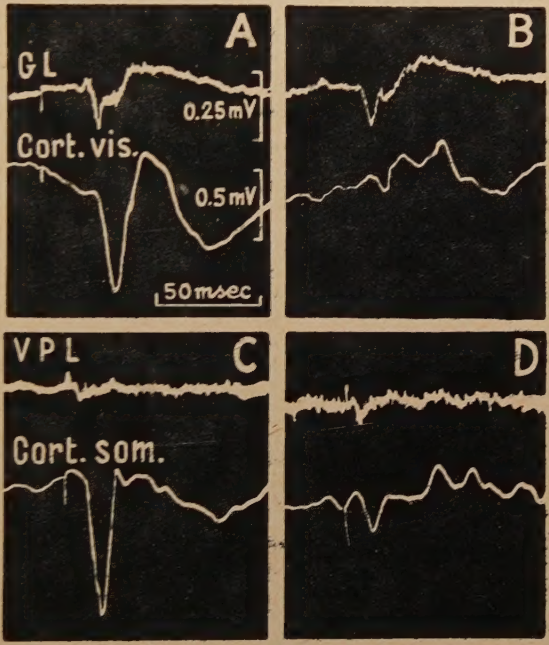

Fig. 4. – *Absence de réduction du potentiel évoqué du noyau de relais thalamique dans l'éveil réticulo-cortical, contrastant avec l'inhibition de la réponse corticale.*

Lapins, immobilisés au flaxédil. *A* et *B*: dérivations du corps genouillé latéral (tracés supérieurs) et de l'aire visuelle correspondante: Réponses à un bref éclair lumineux. *C* et *D*: dérivations du noyau V. P. L. (tracés supérieurs) et de l'aire somesthésique; réponses à un choc électrique sur la patte antérieure contralatérale. *A*, *C*: animal somnolent; *B*, *D*: animal venant d'être éveillé par un frôlement cutané.

lographique n'a été accompagnée d'aucun affaiblissement du potentiel évoqué du corps genouillé latéral (Fig. 4, *A* et *B*). Dans quatre autres expériences la réduction était légère et hors de proportion avec celle du potentiel évoqué cortical. La figure 4 (*C* et *D*) montre le même contraste en ce qui concerne les potentiels évoqués de l'aire somesthésique et du noyau VPL.

Au surplus, il vient d'être démontré par Steriade et Demetresco (36), dont nous pouvons confirmer les observations, que l'effet de l'éveil réticulaire sur le potentiel évoqué photique de l'aire visuelle cesse d'être inhibiteur et devient nettement dynamogénique lorsque la stimulation lumineuse est répétitive et que sa fréquence dépasse 5 éclairs/sec. Une semblable dynamogenèse avait d'ailleurs déjà été nettement mise en évidence par Creutzfeldt et Akimoto (7) dans leur étude microphysiologique de l'interaction, au niveau de l'aire visuelle, d'influx rétiniens et thalamiques non spécifiques.

L'explication de l'inhibition du potentiel évoqué cortical par un processus de convergence occlusive (cf. 37) implique l'hypothèse d'une excitation supraliminaire d'une partie des neurones corticaux par les influx réticulaires ascendants. L'existence d'une pareille excitation supraliminaire n'est généralement pas manifeste dans les tracés corticaux d'éveil obtenus par dérivation macroélectrodique de surface. L'accélération irrégulière et l'aplatissement du tracé électrocortical pourraient en effet résulter simplement d'un processus de désynchronisation, n'impliquant pas une véritable activation des réseaux neuroniques de l'écorce. La réalité de cette activation ressort toutefois de nombreuses données microphysiologiques (28, 33 34, 38). Un aplatissement du tracé cortical n'est d'ailleurs pas l'effet constant de la stimulation réticulaire. Celle-ci peut déterminer une augmentation très nette de l'amplitude des potentiels spontanés en même temps que leur accélération régulière. C'est ce que montrent les tracés *A*, *C* et *D* de la figure 5. Dans l'expérience où ces tracés ont été recueillis, la stimulation réticulaire avait les effets d'éveil habituels: inhibition de la réponse corticale au clic; dynamogenèse de la réponse du même point de l'écorce au stimulus appliqué sur le corps genouillé médian (Fig. 3, *B* et *D*); intensification de tous les symptômes oculaires de l'éveil. On remarquera que l'on retrouve sur les tracés *B* et *D* de la figure 3, enregistrés à une beaucoup plus grande vitesse que ceux de la figure 4, *A*, *C*, *D*, les ondes régulièrement rythmiques et sinusoidales de l'activation réticulo-corticale, précédant le potentiel évoqué.

La synchronisation des potentiels corticaux unitaires pendant l'activation réticulaire a permis de mettre en évidence dans cette même expérience la réciprocité des effets dépressifs exercés par les influx réticulaires et spécifiques sur les potentiels corticaux qu'ils suscitaient. Au potentiel évoqué auditif, réduit ou augmenté, selon le cas, par la stimulation réticulaire, faisait suite régulièrement une dépression transitoire des potentiels corticaux rythmiques de l'activation d'éveil (Fig. 3, *B* et *D*). De pareilles images nous paraissent apporter encore un argument à l'appui de l'explication du « masquage » du potentiel évoqué cortical dans l'éveil par un processus de convergence occlusive.

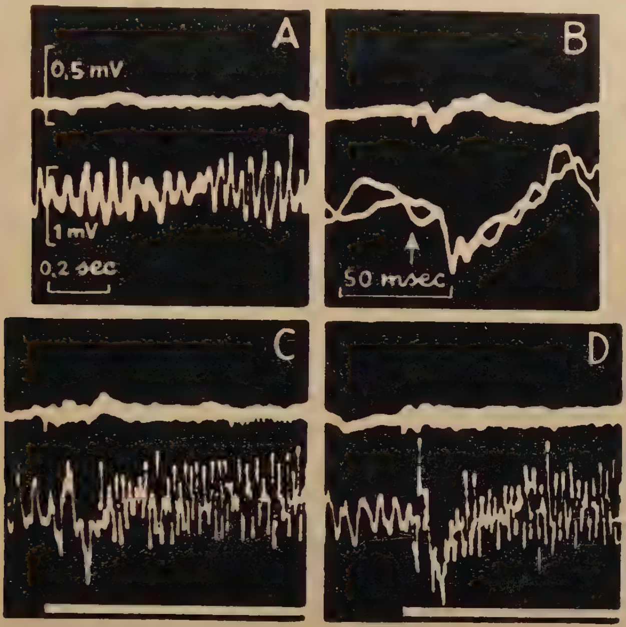

Fig. 5. – ***Exemple d'activation synchronisatrice et amplifiante de l'écorce cérébrale par la stimulation réticulaire d'éveil.***

Chat, encéphale isolé. Même animal que dans celui de la Fig. 3. Dérivations du corps genouillé médian (tracés supérieurs de chaque paire) et de l'aire auditive primaire. *A* : activité spontanée « de repos ». *B* : superposition de deux réponses à un clic. *C* et *D* (le temps est le même qu'en *A*) : activation réticulo-corticale par stimulation de la formation réticulaire mésencéphalique à 50/sec (*C*) et 100/sec (*D*), 2,5 V. Remarquer que l'accélération synchronisatrice met un certain temps pour s'organiser et que la fréquence des ondes de potentiel est finalement la même en *C* et en *D*, malgré la différence du simple au double de la fréquence du stimulus réticulaire répétitif.

Dans notre hypothèse, l'inhibition du potentiel évoqué cortical, lorsque celui-ci est provoqué par un stimulus « périphérique » résulte de la faible puissance de transmission synaptique des influx corticipètes suscités par ce stimulus et de leur incapacité de surmonter la réfractorité relative des interneurones corticaux qui ont été excités supraliminairement par les influx réticulaires d'éveil. Cette situation devrait changer si, par le moyen d'agents pharmacodynamiques, la puissance de transmission synaptique de la volée d'influx suscitée par le stimulus « périphérique » était suffisamment exaltée. C'est

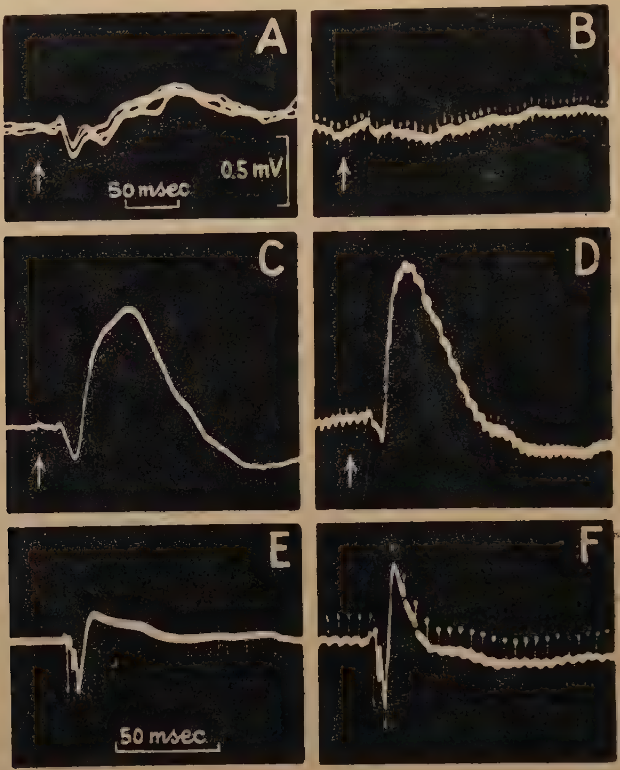

Fig. 6. – *Effet de la strychnisation locale de l'écorce sur le masquage réticulaire du potentiel évoqué cortical.*

Chat immobilisé au flaxédil. Potentiels de l'aire auditive primaire. *A* à *B*: réponses au clic. *A*: animal somnolent; *B*: animal éveillé par une stimulation de la formation réticulaire mésencéphalique (200/sec; 0,3 msec; 2 V). *C* et *D*: effet du même éveil réticulaire (*D*) après la strychnisation locale du point exploré de l'aire auditive. *E* et *F*: effet potentiateur de la stimulation réticulaire sur la réponse au choc genouillé (*F*). Autres explications dans le texte.

effectivement ce qui a été observé dans des expériences où ce stimulus était un bref claquement sonore. La figure 6 montre qu'une stimulation de la formation réticulaire mésencéphalique qui avait régulièrement comme effet une inhibition presque complète de la réponse de l'aire auditive primaire au clic (tracés *A* et *B*) devenait nettement dynamogénique (*C* et *D*) ou cessait d'être inhibitrice après une application locale de strychnine sur la région corticale explorée. Dans cette même expérience, la réponse de l'aire auditive non strychnisée à un stimulus genouillé avait montré la potentiation habituelle (tracés *E* et *F*). Un résultat semblable a été observé à la suite de l'injection intraveineuse de fortes doses (1 à 2 mg/Kg) de sulfate d'atropine. Dans ce cas, la suppression de l'effet inhibiteur de la stimulation réticulaire et son éventuel remplacement par une facilitation pourraient en partie s'expliquer par le ralentissement du tracé électrocorticographique et la suppression de l'activation accélératrice, c'est-à-dire de l'excitation corticale supraliminaire, exercée normalement par les influx d'éveil (cf. 5, 8).

3. *Signification fonctionnelle des modifications de la réactivité corticale dans l'éveil réticulaire.* — L'évaluation de la signification fonctionnelle des aspects électrophysiologiques d'opérations cérébrales est une tâche notoirement difficile. En effet les informations que l'on possède sur la succession des processus neurophysiologiques allant de la réception des influx sensoriels dans l'aire de projection corticale à l'intégration perceptuelle sont encore très fragmentaires (voir 1, 16, 26, 29) et les principes dynamiques de cette intégration sont à peine entrevus. D'autre part, l'établissement de corrélations entre les données psychologiques subjectives et les variations d'amplitude des potentiels évoqués corticaux se heurte à l'impossibilité de l'enregistrement de ceux-ci chez l'homme dans des conditions de précision comparables à celles que permet l'expérimentation sur l'animal, encore que d'intéressantes tentatives dans ce sens, sur lesquelles nous allons revenir, aient été faites récemment.

On en est donc réduit à essayer de déduire une interprétation fonctionnelle plausible des données oscillographiques expérimentales d'une analyse théorique basée sur des principes neurophysiologiques d'une validité généralement acceptée.

L'activation d'une aire de réception corticale par un choc électrique appliqué sur le noyau de relais thalamique correspondant ou sur le nerf optique représente évidemment un mode très artificiel de susciter une opération cérébrale perceptuelle. Aussi, l'augmentation d'amplitude, qui peut être considérable, de ces réponses dans l'éveil réticulaire ne peut-elle être considérée que comme l'indication de l'existence *virtuelle* d'un processus de dynamogenèse, dont il importe alors d'essayer de dégager la signification fonctionnelle.

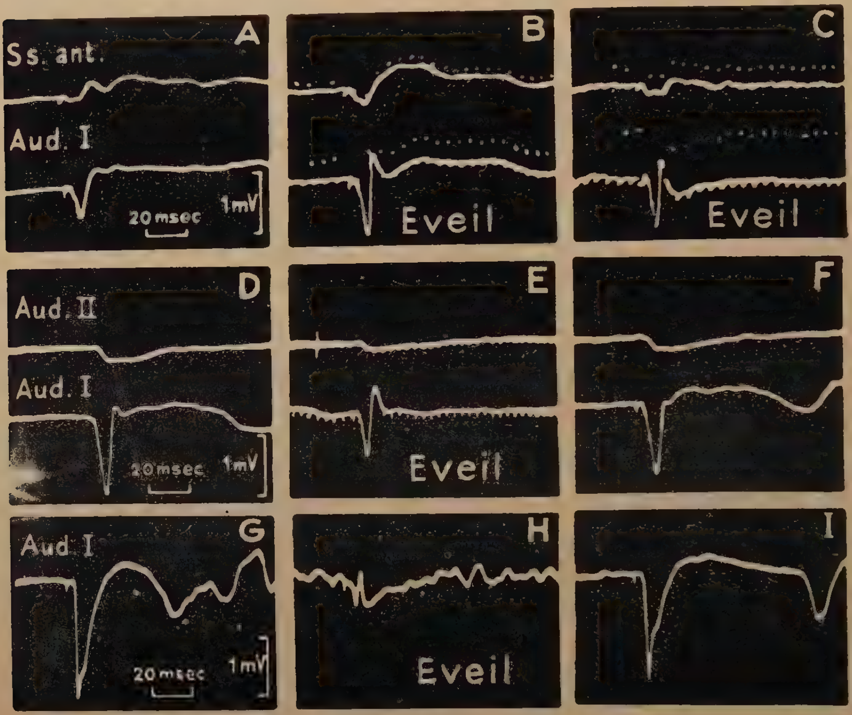

Fig. 7. – *Similitude des profils des réponses de l'aire auditive au choc genouillé et au clic dans l'éveil réticulo-cortical.*

Trois chats, encéphale isolé. *A-C*: réponse de contrôle à un choc genouillé et réponses enregistrées pendant des stimulations réticulaires d'intensité différente. *D-F* et *G-I*: réponses au clic enregistrées chez l'animal somnolent (*D*, *F*, *G*, *I*) et chez l'animal éveillé par une stimulation répétitive de la formation réticulaire (*E*) ou à la suite d'un appel vocal (*H*). Remarquer la similitude de morphologie des réponses *B*, *C*, *E*, *H*. Autres explications dans le texte.

Dans cette recherche, l'étude des modifications que produit la stimulation réticulaire sur le potentiel évoqué de la même aire corticale suscité par la stimulation de récepteurs périphériques est instructive pour deux raisons, l'une de principe, l'autre de fait. La première est qu'il s'agit ici d'un stimulus que l'on peut considérer comme physiologique. La seconde est que l'influence de l'éveil réticulaire sur les potentiels corticaux ainsi évoqués, est comme on l'a vu, très différente de celle qu'exerce le même éveil sur les réponses au stimulus « central ».

En raison de sa régularité et de sa simplicité, la réponse de l'aire auditive à un bref stimulus sonore se prête particulièrement bien à cette étude comparative. A première vue, l'éveil réticulaire ne détermine qu'un affaiblissement plus ou moins marqué de la réponse au clic. Mais lorsque l'on examine les choses de plus près, l'on constate souvent qu'il existe une modification morphologique de celle-ci, caractérisée par l'association, à la diminution d'amplitude du potentiel évoqué, qui est l'expression de la réduction du nombre des neurones corticaux participant à la réaction, d'une accélération de la succession des deux phases lentes constitutives, positive et négative du potentiel. Le profil de celui-ci (Fig. 7, *E*) peut alors, être de forme très semblable à celui de la réponse de l'aire auditive à un choc « genouillé » enregistré pendant une stimulation réticulaire (Fig. 7, *B* et *C*). Dans les deux cas, cette morphologie se caractérise par l'accélération d'ensemble du tracé et par

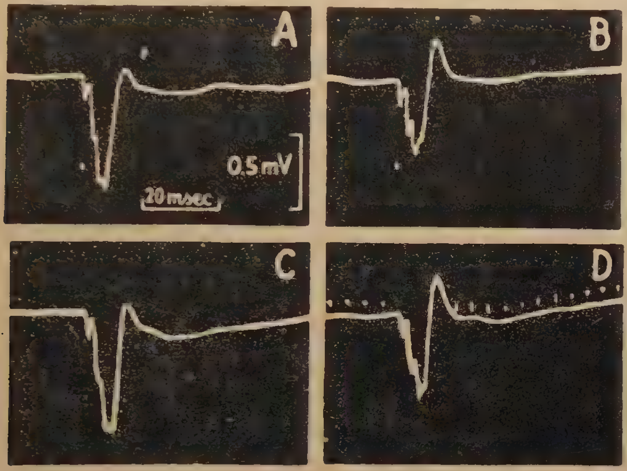

Fig. 8. – *Modifications semblables de la forme du potentiel évoqué cortical dans l'éveil sensoriel et dans l'éveil réticulaire.*

Lapin immobilisé au flaxédil. Réponses de l'aire visuelle au choc genouillé. *A* et *C*: animal somnolent. *B* et *D*: animal éveillé à la suite d'un frôlement cutané (*B*) ou (*D*) d'une stimulation de la formation réticulaire mésencéphalique (200/sec; 0,3 msec; 2 V).

l'accentuation relative de la phase surface-négative de la réponse. Le même fait est visible sur la réponse *H* de la même figure 7, provoquée pendant un éveil sensoriel.

Cette interprétation de l'effet de l'éveil sur la réponse au stimulus périphérique trouve une confirmation dans les résultats d'une étude, faite par l'un de nous (V. R.), des modifications des potentiels évoqués corticaux chez le lapin non narcotisé, curarisé ou non. L'effet de l'éveil obtenu par stimulation réticulaire ou sensorielle a été une réduction d'amplitude très marquée des réponses au stimulus périphérique (comme chez le chat), mais aussi, en général, une réduction légère du potentiel évoqué suscité par un stimulus thalamique. Cependant cette réponse réduite a présenté souvent une modification de son profil très semblable à celle qui vient d'être décrite pour les potentiels évoqués corticaux enregistrés chez le chat (Fig. 8). Sans vouloir entrer ici dans la discussion des différences de comportement des potentiels évoqués corticaux chez les deux animaux, nous mentionnerons simplement que l'état du lapin non narcotisé est caractérisé par une extrême sensibilité aux influx d'éveil, qui le fait ressembler au chat amphétaminisé, dont nous avons montré (5) que les potentiels évoqués, amplifiés par rapport aux réponses de contrôle enregistrées avant l'intoxication, sont paradoxalement réduits dans l'éveil réticulo-cortical.

DISCUSSION

La conclusion que nous croyons pouvoir déduire de cette analyse est que la réponse de l'écorce sensorielle au stimulus appliqué sur les récepteurs périphériques subit dans l'éveil réticulaire une double influence. A la dépression d'amplitude, résultant de la convergence occlusive des influx sensoriels et réticulaires, qui réduit plus ou moins fortement le nombre de neurones corticaux participant à la réponse, sont associés des processus de dynamogenèse tendant à accélerer dans l'écorce les transmissions polysynaptiques qui aboutissent finalement à la phase dendritique du potentiel évoqué. Rappelons par ailleurs qu'il a été observé par Li (27) que, pendant l'oblitération plus ou moins complète du potentiel évoqué sensoriel recueilli par une macroélectrode superficielle, la décharge axonique unitaire d'un neurone cortical sous-jacent à cette électrode peut être nettement intensifiée sous l'influence de l'interaction d'une volée d'influx non spécifiques (réticulaires thalamiques), conditionnant la réponse à la volée spécifique.

Dans cette perspective, l'action des influx réticulaires ascendants sur les opérations élémentaires des réseaux neuroniques corticaux pourraient acquérir la signification d'une sélection probabiliste. L'activation réticulo-corticale éliminerait de la réponse aux influx spécifiques un certain nombre de neurones corticaux. Mais, en même temps, elle renforcerait, par un processus de convergence facilitatrice, le pouvoir de transmission thalamo-corticale des influx sensoriels les plus spécifiques en ce qui concerne l'aire réceptrice considérée et augmenterait les irradiations intracérébrales des neurones excités par ces influx. La prépondérance de l'effet inhibiteur, dans le cas d'activation réticulo-corticale très intense, rendrait compte de l'obtusion sensorielle qui caractérise les états d'extrême excitation émotionnelle et qui chez l'animal s'exprime par une « arrest-reaction » ou une immobilisation d'allure catatonique (21, 2).

Une difficulté de toute interprétation des processus de l'éveil réticulo-cortical basée sur l'hypothèse, par ailleurs si plausible, de la convergence des influx réticulaires et des influx spécifiques sur des interneurones communs est qu'elle implique à première vue que les influx réticulaires devraient être capables, à eux seuls, de produire les activations des réseaux neuroniques corticaux qui sont le substratum de la sensation. Un éveil réticulaire intense devrait, dans ce cas, être hallucinogène. Mais nous avons montré antérieurement (4) que cette déduction ne s'impose pas nécessairement et indiqué par quelle organisation synaptique les influx ascendants réticulaires pourraient, tout en étant puissamment dynamogénisants pour les réponses aux influx spécifiques, ne jamais atteindre le seuil réactionnel des esthésioneurones sensoriels corticaux (cf. 2).

Le caractère conjectural de cette interprétation fonctionnelle des phénomènes qui caractérisent l'éveil réticulaire au niveau thalamo-cortical reflète l'indigence de nos connaissances en ce qui concerne les processus de l'intégration perceptuelle.

Les quelques observations psychophysiologiques dont on dispose révèlent la complexité de la relation entre la réponse subjective ou comportementale et l'amplitude du potentiel évoqué cortical suscité par une volée d'influx afférents. Hernandez-Péon et Donoso (17) ont noté sur des sujets humains une corrélation positive nette entre le voltage du potentiel évoqué enregistré dans les radiations optiques et l'intensité de la sensation visuelle suscitée par un bref stimulus photique[1]. Rosenblith (32) a constaté d'autre part que le seuil absolu de la sensation auditive provoquée par un clic s'abaissait chez le sujet légèrement somnolent, dans une condition par conséquent où il y a lieu de présumer que l'amplitude du potentiel évoqué cortical est plus grande que dans l'éveil.

Par contre, lorsqu'il s'est agi d'opérations cérébrales d'un niveau plus élevé, impliquant discrimination, motivation et choix, l'intensification de la vigilance par stimulation réticulaire s'est avérée être nettement facilitatrice. Fuster (12, 13) a fait sur le singe, porteur en expérience chronique d'électrodes intraréticulaires, l'observation significative que la reconnaissance correcte d'objets divers présentés tachistoscopiquement était plus rapide pendant un éveil réticulo-cortical que dans les conditions de détente sensorielle et de somnolence. Lansing, Schwartz et Lindsley (25) ont constaté d'autre part que le temps de réaction visuomoteur, mesuré chez l'homme, était nettement raccourci quand un avertissement préalable avait eu comme effet une désynchronisation électroencéphalographique, témoignage d'un état de veille attentive.

Si l'on tient compte du niveau plus ou moins élevé des opérations perceptuelles impliquées dans les tests utilisés, il nous semble que ces observations sur l'homme et sur l'animal, pour fragmentaires qu'elles soient, ne sont pas en désaccord avec l'interprétation proposée des modifications des potentiels évoqués corticaux, et que leur confrontation avec les données expérimentales est susceptible d'éclairer la signification psychophysiologique des effets de l'éveil réticulo-cortical.

RÉSUMÉ

L'étude, sur le chat encéphale isolé, de l'interaction de la dynamogenèse réticulaire du potentiel évoqué cortical (visuel) avec d'autres mécanismes de facilitation de celui-ci a montré que ces différentes dynamogenèses ne sont pas additives ou ne le sont que très partiellement. Ce fait peut s'expliquer par leur utilisation commune du contigent limité de neurones qui constitue la frange sous-liminaire de la réponse au stimulus non conditionné.

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[1] Le docteur Jouvet nous fait savoir qu'il n'a pu observer cette corrélation dans des expériences semblables de stimulation photique, mais que par contre elle a été nette dans une expérience de dérivation de la réponse somesthésique thalamique à un stimulus tactile (disparition du potentiel et de la sensation pendant l'effort d'un calcul mental). « Il semble », écrit-il, « que l'attention soit une des conditions de la perceptivité du signal et que l'intensité subjective appartienne à un autre domaine mettant en jeu probablement une mémorisation et une intégration très complexe ».

Il a été constaté par contre que le stimulus réticulaire est encore capable d'augmenter fortement le potentiel évoqué d'une aire de réception, déjà très amplifié à la suite de la strychnisation locale de cette aire. Tout se passe comme si la strychnine, en étendant considérablement la frange sous-liminaire de la réponse, augmentait proportionnellement les possibilités de recrutement neuronique par les influx réticulaires ascendants.

L'analyse de l'effet d'inhibition exercée par la stimulation réticulaire d'éveil sur la réponse de l'aire auditive corticale à un bref stimulus sonore a permis d'apporter de nouveaux arguments à l'appui de l'explication du phénomène par un processus de convergence occlusive entre influx réticulaires et influx spécifiques. Elle a mis en évidence d'autre part l'association, à une modification paraissant purement inhibitrice, d'un élément de dynamogenèse qu'il est possible de favoriser expérimentalement.

La signification fonctionnelle des diverses modifications des potentiels évoqués corticaux dans l'éveil fait l'objet d'une discussion basée sur les faits expérimentaux et sur des données de la littérature psychophysiologique.

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UNITARY ANALYSIS OF THE RESPONSE ELICITED IN THE VISUAL CORTEX OF CAT[1]

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Introduction

The interpretation of the various components of the specific cortical response produced by electrical stimulation in the visual pathways and first described in detail by Bishop and O'Leary (5), is still a matter of controversy. Although, in its typical from, it is characteristic of the visual cortex, similar complex evoked potentials have also been described for other specific sensory areas in special experimental conditions (21).

Such a response, unelicitable by physiological stimuli, is undoubtedly artificial, and one may wonder whether it is worth making it the object of a detailed study. On the other hand it may be considered a useful tool which lends itself to the analysis of basic properties of the specific projection systems. In view of the fact that far-reaching conclusions concerning their organization have been built upon the functional substrate of this response, further investigations appear justified.

The response consists of four brief positive deflections followed by a less constant slow negative wave. There is an almost general agreement that the first wave represent the action potential of radiation fibers and that waves 4 and 5 are the expression of intracortical, post-synaptic activation. As far as the nature of waves 2 and 3 is concerned, however, the opinions of various authors markedly differ. Bishop *et al.* (3, 4, 5) consider both of them as manifesting activation of post-synaptic elements within the cortex, namely interneurons of Golgi type II (wave 2) and pyramidal cells (wave 3). Generally similar conclusions are reached by Marshall *et al.* (24) and Purpura and Grundfest (28). According to Schoolman and Evarts (29) the nature of wave 2 is obscure while they agree on the post-synaptic (intracortical) origin of wave 3. Chang (11, 13, 14) on the other hand feels that all the first three waves are pre-synaptic extracortical potentials probably representing three fiber systems of different conduction velocity. Chang's findings to support his theory were not confirmed by other investigators: Malis and Kruger (22) present evidence for the existence of two fiber systems and conclude that only waves 1 and 2 represent activity in the geniculo-striate part of these systems, while the third wave is considered as cortical post-synaptic. This opinion is shared by Grützner *et al.* (18). Bremer and Stoupel (7, 8) agree with Chang (11, 13, 14) on the pre-synaptic nature of the first three waves but feel that waves 2 and 3 are due to intracortical fibers in which an abrupt tapering of their terminations has taken place with a consequent slowing of conduction velocity.

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The purpose of the present work is to study temporal and spatial distribution of unit activity within the visual cortex and underlying white matter during the course of the evoked surface response, and to analyze the functional characteristics of these unitary elements in relation to its various phases. We hope, through such a close correlative study, better to understand the nature of the various components of the response.

A microelectrode analysis of the cortical response to electrical stimulation of the visual pathways has recently been performed by Grützner *et al.* (18). In their very thorough and careful study, an attempt to correlate unit activity with the surface response was also made. Their method, however, differs from ours in that they stimulated the optic nerve instead of the radiations: the geniculate relay being interposed between stimulating and pick-up electrode, all their units were "post-synaptic" (see below) and few of their results and conclusions are directly pertinent to the present study.

METHODS

Experiments were performed on cats in light nembutal anesthesia (20-30 mg/kg given intravenously before the operative procedure; later only small amounts of nembutal being added if necessary). The cerebral cortex was widely exposed bilaterally and the cisterna magna opened. The exposed nervous structures were covered with paraffin oil. The stimulating electrodes were orientated with a stereotaxic instrument according to the coordinates given by Jasper and Ajmone-Marsan (20). For identification and analysis of cortical units it was felt necessary to stimulate the optic radiation thus avoiding inteference with the synapses of the lateral geniculate nucleus. For special purposes, however, *e. g.*, to decide whether a given recorded spike was picked up from corticopetal or corticofugal fibres, optic tract or nerve stimuli were used, in some experiments, alternated with radiation stimuli. The radiation was stimulated close to but above the lateral geniculate, carefully avoiding stimulation of presynaptic elements of this nucleus (cf. 21, 29).

Two Grass stimulators were used. The duration of the square waves was kept between 0.1 and 0.5 msec and their voltage adjusted for subliminal, threshold or supramaximal effects as needed. The responses were recorded from the middle portion of the horizontal part of the gyrus lateralis. The frame of the stereotaxic instrument served as indifferent electrode. Conventional silver ball electrodes were used for recording from the surface of the cortex within 1 mm distance from the point of insertion of the microelectrodes. The latter were of the type described by Hubel (19), *i. e.*, electropolished tungsten wires, coated with a vinly lacquer Their tip diameter is 1-2 μ, and, tested in saline with a frequency of 1000 c/sec, their impedance is about 10 MΩ. With these electrodes, intracellular recording is exceptional: most spikes, if not all, have been recorded extracellularly and it is therefore difficult at times to know if one records the activity of only one unit. The usual criteria have been used in this study and in the detailed analysis of eventual spike changes, doubtful spikes have been discarded. For the main purpose of this work, however, the possibility of simultaneously recording spikes from more than one unit is of little concern. Due to the relative coarsness of the electrodes it is also probable that the activity of the smallest elements could not be picked up.

The microelectrode was connected to a cathode follower input circuit (2). Conventional AC-coupled amplifiers with a time constant of 1 sec were used. In all tracings, positivity is expressed by upward deflection for the microelectrode and by a downward deflection for the gross surface electrode pick-ups. A micrometer drive, connected to a hydraulic system was employed for the advancement of the microelectrode at steps of approximately 1.5 μ. The microelectrode was inserted through the arachnoidea perpendicularly to the surface and in the middle of the gyrus to avoid the folded parts of the cortex. Only in a few cases was the position of the microelectrode tip checked histologically. In the remaining cases the exact localization cannot be determined. Considering the errors involved in the method it is obvious that the values obtained from the micrometer drive are only approximate and in single cases might even be misleading if the track of the electrode was accidentally oblique. For that reason the statements made in this paper as to the localization of units should be regarded only as rough estimates. A unit has been considered to be located in the white substance only when the micrometer has shown values well above 2 mm in depth. No conclusions about localization have been drawn from single observations but from the general trend in all the experiments (see *e. g.*, Fig. 2). No recording was done during retraction of the microelectrode.

The various components of the surface response (Fig. 1) have been labelled according to the generally adopted convention (see *e. g.*, 29). Only

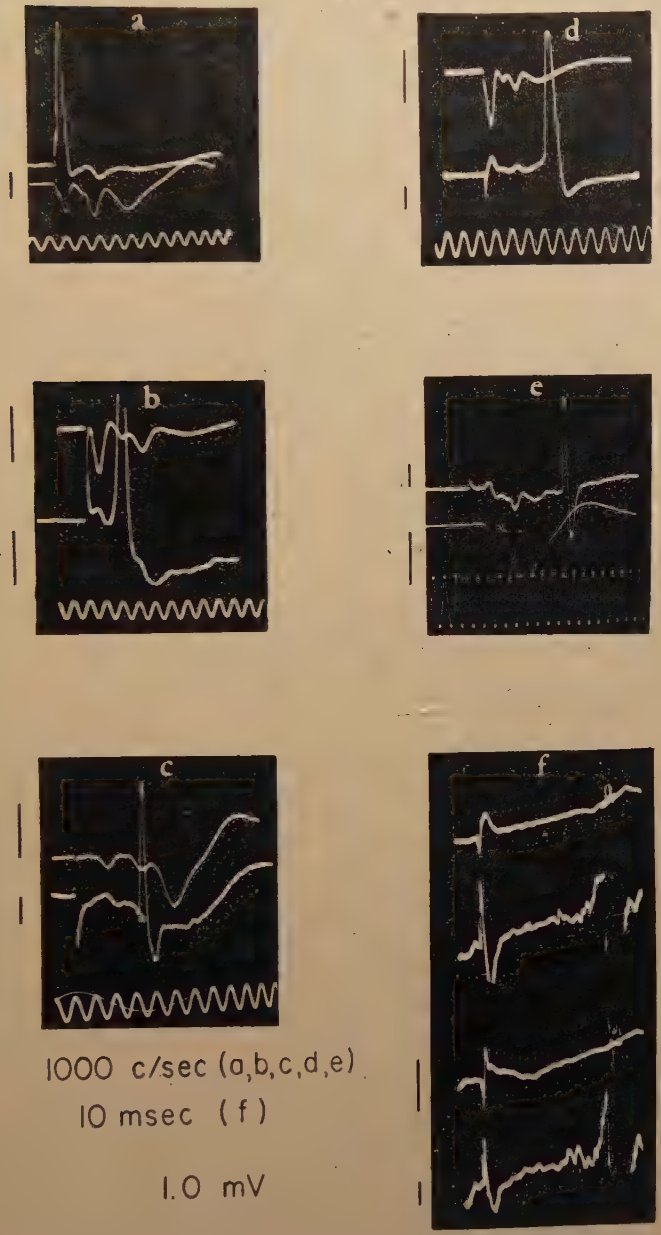

Fig. 1. – *Various examples of visual cortex response showing the activation of different unit spikes.*

The various spikes are temporally related to each of the five components of the surface evoked potential (*a-e*) or firing late after its end (*f*). (Microelectrode tracing in lower channel in *b-f*; in upper channel in *a*).

Chang and Chang and Kaada (11-14) have used another nomenclature, labelling as "wave 1" a small negative deflection preceding the first positive wave (this is seen only when stimuli are applied to the optic nerve or tract). Measurements of latencies and peak amplitudes of the waves were made in relation to the ideal baseline. This method is not unobjectionable but was also employed by Bishop and Clare (3) and we failed to find a more satisfactory one. When correlating the results of different experiments, latencies of unit discharges were normalized to those of the corresponding component of the slow surface response to correct for unavoidable variations (see Fig. 1).

RESULTS

1. *Types of units and morphology of spikes.* — Unit spikes recorded under the present experimental conditions may be divided into four different groups (see also 18): I) activated by the stimulus and firing during the course of the primary visual response; II) responding with a long latency after the end of the surface response (Fig. 1 f); III) "spontaneously" active but uninfluenced by the stimulus; IV) "spontaneously" active and inhibited by the stimulus. The majority of recorded spikes belong to group I; only these, which are of interest to our problem, were analyzed in detail and any one of them may be temporally correlated with one of the five components of the surface response (Fig. 1).

Morphological details concerning the spikes are as follows: the majority has an initial positivity; some are monophasic, other diphasic positive-negative. The few initially negative spikes observed were small and very likely the expression of distant pick-up. Spikes may remain essentially unchanged for electrode displacement of 25 to 50 μ and with more than 100 μ movements the spike is not necessarily lost (this fact would suggest that the brain tissue may be pulled downward with the microelectrode). Usually, spikes recorded with this method have no characteristics which make it possible to identify the active structure (e. g., axon or cell body). Sometimes, however, the spike originates from a slow graded "prepotential" probably due to the synaptically initiated depolarization (Fig. 12), in which case the microelectrode tip must be close to the soma. In other instances, with high frequency repetitive stimuli or with closely spaced, paired stimuli, it has been possible to provoke an inflection on the rising limb of an action potential demonstrating that the spike in these cases consists of two different components (Fig. 6). Because of the similarities between these and intracellular records from cell bodies, it seems justified to consider such potentials as derived from somata. Radiation axon spikes recorded from white substance are always initially positive (see also 31).

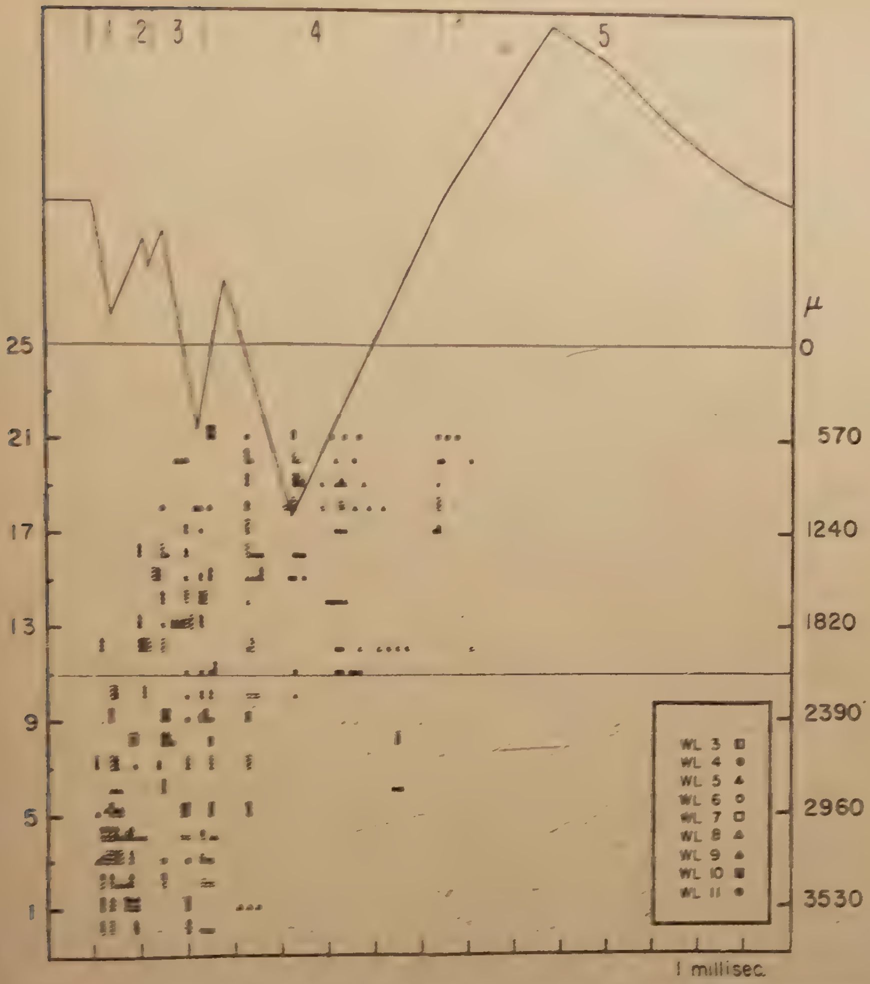

Fig. 2. – *Plotting of 135 unit spikes recorded in 9 different experiments.*

Along the abscissa they are in temporal relationship with a prototype of surface response. Latency values are not absolute but each unit spike has been correlated to the actual response simultaneously recorded and its relationship has been preserved and plotted in relation to the schematic response, onset and peak of its various phases serving as reference points. A spike may thus be related to the onset or peak, or to the interval between onset and peak or peak and end of each given of the 5 waves. Due to the method adopted for estimating the starting points of the surface waves, some overlapping is unavoidable: when a spike occurs close to the terminal phase of one wave and to the initial phase of the following one, it has been plotted as related to the latter. For every unit 3 spikes are plotted: if their latency was stable, the symbols are in a straight vertical line, if it varied they appear in a horizontal line demonstrating the maximal range of variation. Along the ordinate, the spikes are plotted in relation to depth (micromanipulator readings on the left, extrapolated microns on the right), the two horizontal dotted lines outlining the approximate thickness of the cortex.

2. *Spatial and temporal distribution of the units.* — Examples of unit firing in correspondence with the various components of the surface response are illustrated in Fig. 1 *a-e*.

In Fig. 2, spikes from 135 units recorded in 9 different experiments are plotted. As shown by the figure, there is a trend for deep spikes to correspond to the early phases of the surface response. This is particularly true for those which are picked up very deeply, and unquestionably from the white substance. More superficially, on the other hand, spikes recorded during the 1st wave of the surface response are very rare or absent. In correspondence to the 3rd wave there are discharging units both in the deepest and most superficial levels explored; while in correspondence to the 4th wave, particularly its late phase, the units are predominantly found within the cortex. Units firing during the large 5th wave were exceptional in the experiments used for the plotting and in general are relatively rare and found only during its initial part.

3. *Classification and functional characteristics of the units.* — According to their behaviour following optic radiation stimulation, all unitary spikes may be divided into post-synaptic and pre-synaptic.

a) *The post-synaptic group.* Spikes in this group are quite sensitive to repetitive stimulation and are usually completely blocked by stimuli of a frequency of about 10-20/sec. Tested with paired stimuli, these units show longlasting periods of unresponsiveness after a conditioning shock. The duration of this effect varies with the strength of the conditioning stimulus but can always be made to last 10 msec, usually much longer. These spikes often show in addition spontaneous variations in latency of 0.5 msec or more and in probability of discharge.

b) *The pre-synaptic group.* Spikes belonging to this group are considered to originate in optic radiation fibers and/or their intracortical extensions. They are quite stable, the stimulus-response ratio being 1: 1 for suprathreshold strength, with no or negligible spontaneous variations in latency. With repetitive stimuli, the stimulus-response ratio is unchanged for frequencies of up to 200-300/sec; higher frequencies have not been tried. After a supraliminal conditioning stimulus, these units respond to a test shock if this is given with a delay exceeding the duration of the spike potential.

The validity of the criteria used in this classification is discussed later.

4. *Differential behaviour of units related to different waves of the evoked response.* — Units firing during different phases of the surface response were found to have different characteristics. Figs. 3 to 8 illustrate the behaviour of diverse units in double shock experiments or during high frequency stimulation. In Fig. 3 (*f*-*i*) there is a unit, discharging through the first wave of the surface response, recorded from the white substance at a depth of about 3000 μ and with a latency to the foot of the spike of 0.3 msec. When stimulated with double shocks at decreasing shock intervals, the unit is completely refractory to the second shock at intervals of 1.4 msec or less. For intervals of 1.45 to 1.5 msec the test stimulus evokes a spike of lower amplitude than the spike of the conditioning response [1]. In another experiment (Fig. 4), the stimulus evoked a double spike discharge, the first spike corresponding to the first wave and the second spike to the third wave of the surface response. The stimulus threshold for the second spike was somewhat higher. In the double shock experiments illustrated (*B*-*D*) the shock interval was kept

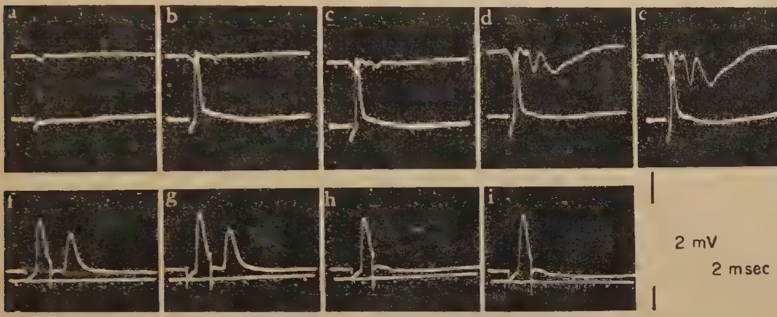

Fig. 3. – *Spike temporally related to wave* 1 *of the visual response.*

a-*e*: Short latency spike: stimulus strength progressively increased from *a* (subliminar) to *e* (supra-maximal). (Microelectrode tracing in lower channel; cortical response in upper channel). *f*-*i*: Behaviour of the same unit with double-shock stimulation. The shock interval is progressively decreased. There is complete refractoriness for intervals of 1.4 msec or less (*h*, *i*). (Note different sweep and gain; microelectrode tracing only). See also Fig. 4.

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[1] Similar reduction in amplitude during rapid firing of a single unit has been reported by several authors, among others by Freygang (16) who recorded intracellularly from axons of the lateral geniculate body. It is thus in no way incompatible with the assumption that the spike is derived from a single unit.

constant at about 8 msec, whereas the strength of the conditioning stimulus was decreased so that in *C* it was sub-threshold for the second spike. The first spike of the test response was apparently uninfluenced by the conditioning stimulus (and this was true also for much shorter shock intervals) but the second spike was absent in the test response if elicited by the first shock. This blocking effect on the second spike was longlasting and is demonstrated in *F* at a shock interval of 30 msec. In agreement with the preceding comments, the second spike is considered a delayed post-synaptic discharge. The first spike of Fig. 4 as well as the spike of Fig. 3 on the other hand, are considered pre-synaptic, *i. e.*, fiber action potentials, likely in the white substance, which have not been relayed through any synapse on their passage from stimulating to recording electrodes. All first wave spikes thus far analyzed have shown similar

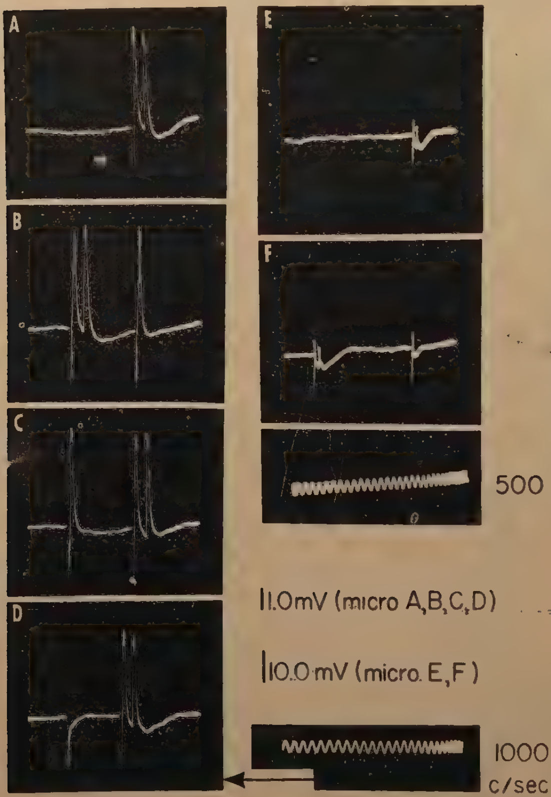

Fig. 4. – *Spike temporally related to wave 1 of the visual response.*

A, E: Test response alone. The stimulus evokes a double discharge. *B-D*: Double-shock stimulation. The shock interval is kept constant (about 8 msec) but the strength of the conditioning stimulus is progressively decreased. *F*: The shock interval is increased to about 30 msec. Note longlasting "blocking" effect on second spike of test response (see text for details). (Only the microelectrode tracing is shown. The tracing for the cortical response was originally kept purposely faint and subsequently deleted from the final reproduction).

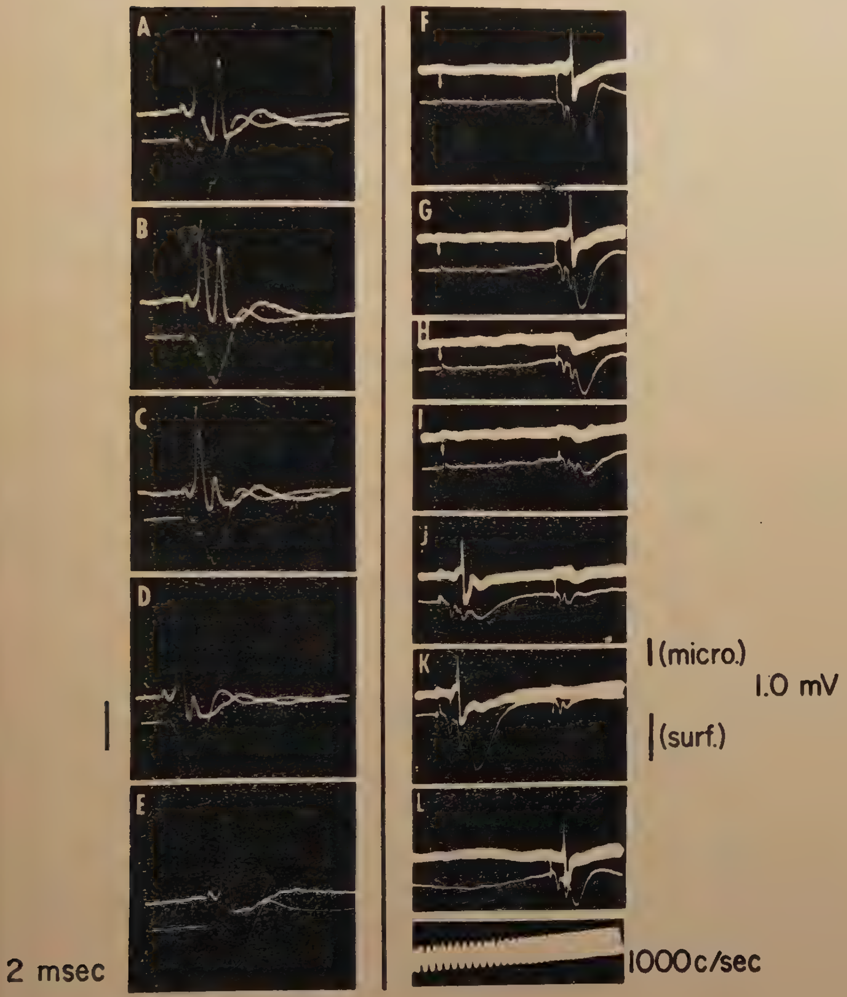

Fig. 5. – *Two types of spikes (A-E and F-L) temporally related to wave 3 of the visual response.*

E, L: Test stimulus alone. *A-D*: Activation of unit with double shocks at decreasing intervals. Note that the test spike still fires, though its amplitude is markedly decreased, when the shock interval is approximately 1.75 msec. *F-K*: Double shock experiments with relatively long intervals between the two stimuli. In *J* and *K* the conditioning stimulus is suprathreshold, and in *F-I* subthreshold for the spike. (See text for details). (Microelectrode tracing in upper channel, cortical response in lower channel). See also Fig. 6.

characteristics being able to discharge twice at short shock intervals down to 1 to 1.5 msec, *i. e.*, with the second shock falling on the descending limb of the preceding spike, and to follow high frequency stimulation. This is true also for the few spikes that have been recorded during the second, short wave of the surface response.

Units discharging during the third wave are of two different types, illustrated in Fig. 5. In *A* through *D* the unit is activated with double shocks at decreasing intervals. In *D*, when the test shock is given about 1.75 msec after the conditioning shock, the unit still discharges though with very much decreased amplitude. This interval is slightly shorter than the duration of the recorded spike potential, corresponding fairly well with the expected duration of the absolute refractory period. Thus, this spike behaviour recalls that of the first wave spike of Fig. 4.

Quite different is the behaviour of the other unit (Fig. 5 *F*-*L*) the action potential of which is shown alone in *L* and in double shock experiments in *K* through *F*. In *K* and *J*, the conditioning stimulus is suprathreshold for the spike, and in this situation the spike of the test response is blocked in spite of the shock interval being as long as 17 msec. This spike was also sensitive to increased frequency of stimulation and was blocked when the frequency exceeded 7-10/sec. It seems logical to conclude that this unit is a post-synaptic one.

The two unit behaviours illustrated in Fig. 5 are representative of most of the spikes temporally related to the third surface wave. These actually may be divided into two groups, one with pre- the other with post-synaptic characteristics, the latter being more common.

Exceptionally, third wave units were found whose classification is not immediately apparent. The spike potential of one of such units is shown in Fig. 6. In *b* and *c* it is conditioned by a stimulus, preceding by 1.2 to 1.3 msec the test shock. The test spike may discharge but it shows not only a lower amplitude, but also a longer latency and a change in shape: an inflection is clearly appreciable on its rising phase and its total duration increases. This inflection was apparent at stimulus intervals below 3 msec. A more gradual evolution of these changes is demonstrated in *d* through *i* where the unit was activated by repetitive stimuli of increasing frequency. A clear inflection was first seen with a frequency of 20 stimuli/sec (*f*), at about 40% of the voltage value of the spike potential,

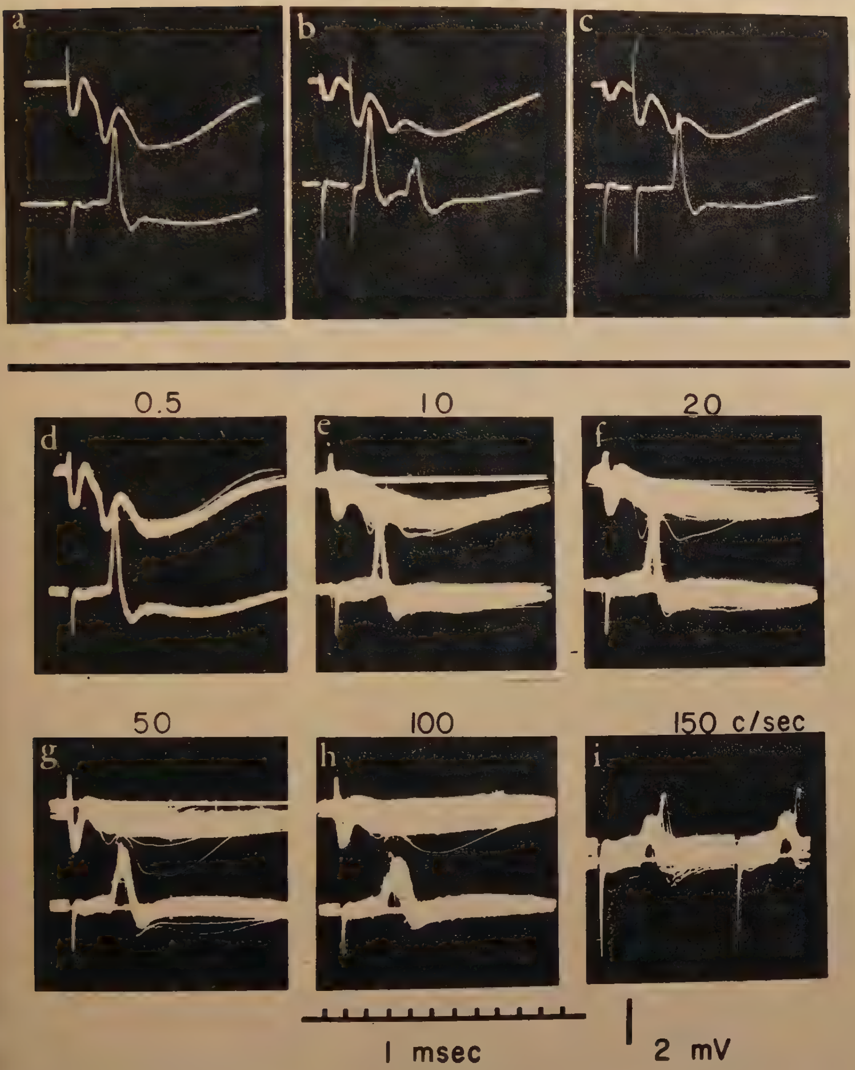

Fig. 6. – *Spike temporally related to wave 3 of the visual response.*

a: Test stimulus alone. *b, c*: Double shock experiments with conditioning stimulus at threshold (*b*) and subthreshold (*c*) strengths for the spike. Note in *b* the test spike present but lower in amplitude, longer in latency and showing an inflection in its rising phase. *d-i*: Same spike. Repetitive stimulation at increasing frequency (superimposed frames). (See text). (Microelectrode tracing in lower channel, cortical response, not shown in *i*, in upper channel).

and it became more and more marked with increasing frequency of stimulation. The two portions of the spike might be compared to the "A" and "B" components of Frank and Fuortes (15, 17) [1]. If this comparison is justified, the inflection would correspond to an

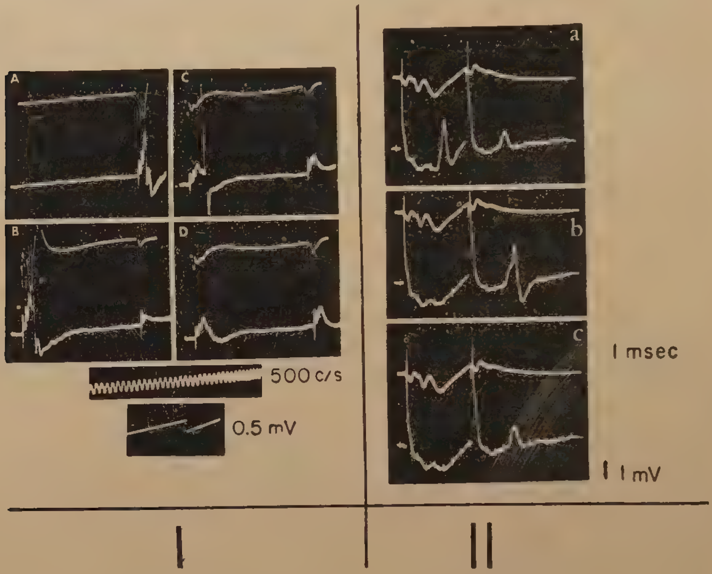

Fig. 7. – *Spikes temporally related to wave 4 of the visual response.*

I. *A*: Test stimulus alone. *B-D*: Double shock experiments; the stimulus interval is unchanged (45-50 msec) but the strength of the conditioning stimulus is progressively decreased. Note longlasting unresponsiveness of the test spike regardless of presence or absence of the conditioning one. II. Double shock experiments. In *a* the conditioning stimulus is supraliminar and in *b*, *c* subliminal for the spike. (See text). (Microelectrode tracing in lower channel; cortical response in upper channel). See also Fig. 8.

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[1] It has been demonstrated with the intracellular technique (9, 10, 15, 17) that the soma potential consists of more than one component, and that the point of transition between them can be more clearly demonstrated when the unit is activated by paired or repetitive stimuli. Such changes have not been found in the action potentials of axons (15). Studying cortical Betz cells, Phillips (27) states that " not only intracellular records but also the more easily obtained extracellular records can be used for the purpose of identifying the two main components of the cellular response ". On the basis of these findings, it is safe to conclude that the action potential of Fig. 6 is a soma spike. This conclusion is given further support by the fact that in all our esperiments in which the tip of the microelectrode was very probably in the white substance, we have never recorded spikes of this composite type.

axon-soma delay of the spread of excitation and in fact some impulses may apparently fail to invade the soma at the highest frequencies used (see Fig. 6 *h*, *i* where the descending phase of the " A " component in several sweeps is uninterrupted by the take off of the " B " component). An inflexion in the rising phase of the spike potential is present not only with antidromic but also with orthodromic activation (see 1, 15) and this has also been found in the present investigation (Fig. 7, II). The fact that the unit of Fig. 6 is able to discharge twice to double shocks of 1.2 to 1.3 msec interval, and to follow repetitive stimuli of a frequency of more than 150/sec suggests that it is a directly activated cell. On the other hand the increase in latency when it discharges to the second of two paired supraliminal stimuli, (though failing to show any abrupt change when plotted versus the duration of shock interval) could suggest its being a post-synaptic, orthodromically activated unit.

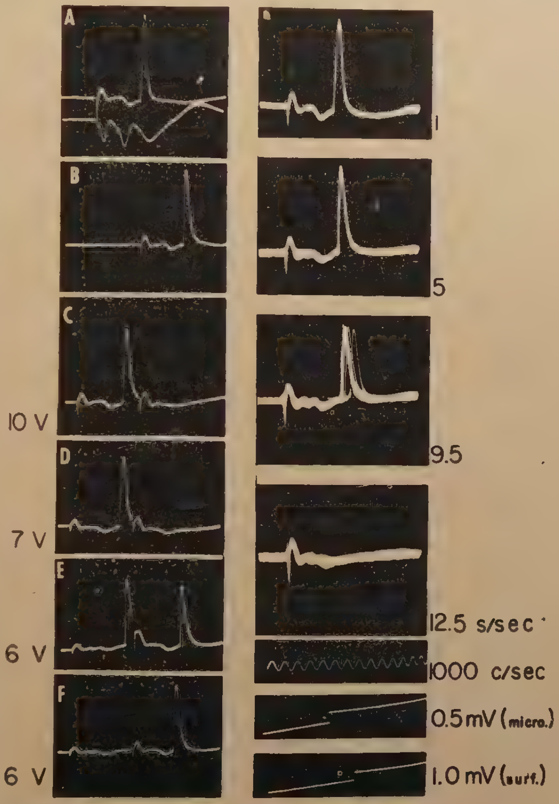

Fig. 8. – *Spike temporally related to wave 4 of the visual response.*

A, *B*: Conditioning and test stimuli alone (slightly higher amplification in *A*). *C-F*: Double shock experiments. The strength of the conditioning stimulus is indicated on the left side of the records; the interval between the two shocks is kept constant at 5 msec. (See text). Right column: Superimposed frames. Repetitive stimulation at progressively increasing frequency, as indicated on right side of records. (Cortical response only shown in *A*, lower channel).

In contrast to units temporally related to wave 1, 2 and sometimes 3, those related to waves 4 and 5 never behave like presynaptic ones. The 4th wave spike of Fig. 7, I, shown alone in *A*, is preceded in *B* by a conditioning shock of the same strength at an interval of 45-50 msec. In *C* and *D* this interval is kept constant but the strength of the conditioning shock is successively decreased, so that in *D* it is subliminal for the spike. There is no unit discharge in the test response, regardless of whether the spike is present or absent in the conditioning response. Thus, after the conditioning shock, there is a longlasting unresponsiveness which cannot be explained as refractoriness of the unit studied.

Occasionally it is possible, by critically adjusting the relative strength of the stimuli, to make a 4th wave unit discharge twice at rather a short shock interval. The unit of Fig. 8, shown in *A* with somewhat higher amplification than in *B-F*, does not respond to the test shock when the strength of the conditioning shock is 7 V or more (*C* and *D*) but when it is decreased to 6 V (just liminal for the " conditioning " spike which may or may not be elicited) the spike of the test response discharges (*E*, *F*). The shock interval was kept constant at 5 msec. It is evident that the ineffectiveness of the test shock in *C* and *D* is not due to refractoriness. In the right column of Fig. 8, the frequency sensitivity of this post-synaptic unit is demonstrated.

The two phenomena illustrated respectively in Figs. 7 and 8; namely: " inhibition " of a test spike by a subliminal conditioning shock, and the " inhibition " of a test spike by increasing the conditioning strength above the threshold value for the spike, have been valuable signs of the post-synaptic character of units, frequently made use of during the course of these experiments.

The " inhibitory " influences on a unit of post-synaptic type by a preceding stimulus to the optic radiation below the threshold for the unit studied are manifested not only as complete failure of discharge, but sometimes as changes in the shape of the spike potential suggestive of delayed spread of excitation over the soma. In Fig. 7, II a 4th wave unit is activated by paired stimuli with an interval of 7-9 msec. In *a* the conditioning stimulus is supraliminal, in *b* and *c* sub-threshold for the unit. In both cases the test spike shows either an inflection on the rising phase or a failure of discharge of the " B " component.

Some further elucidation of the inhibitory mechanism of the visual cortex brought about by a stimulus of the optic radiation is given by the experiment of Fig. 9, where a unit " spontaneously " active in an injury discharge also fires in response to an optic radiation shock. When the stimulus is simultaneous with a " spontaneous " discharge, falling in the refractory period, it fails to evoke a spike (*A*). The unit on the other hand is inhibited for a while after the stimulus (*B*) which fact cannot be explained as refractoriness, neither as the result of anti- or orthodromic excitation of its own recurrent collaterals, as the unit was capable of firing with much higher frequency in the absence of stimulation. In *C* the situation immediately after the " death " of the cell is shown: no spike but a deep negativity is recorded[1].

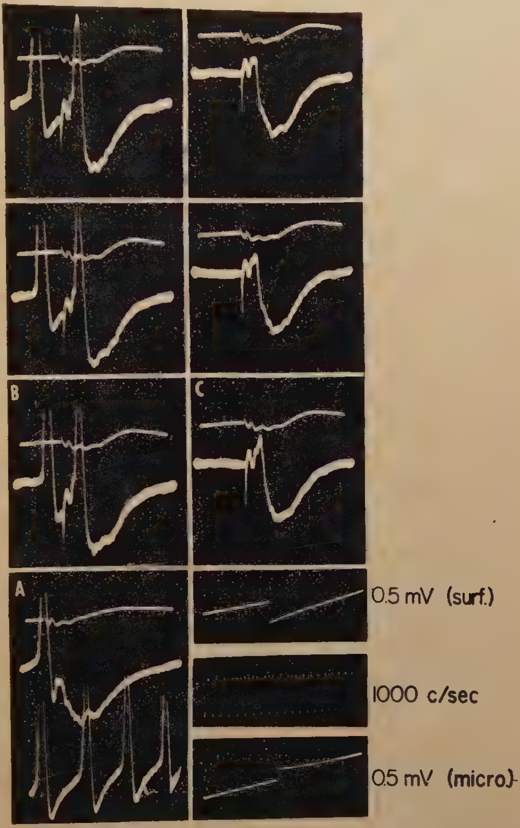

Fig. 9. – *Injury discharge.*

A: Radiation stimulus, simultaneous with a " spontaneous " injury discharge, fails to evoke a spike. *B*: Three records showing an arrest of firing following the spike induced by the stimulation. *C*: Three records showing the effects of stimulation immediately after the cell " death ". Note absence of spike and presence of slow negativity. (See text). (Microelectrode tracing in lower channel; cortical response in upper channel. In *A* the bottom record shows the injury discharge in the absence of electrical stimulation).

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[1] This potential is similar to the negative " swing " in damaged cells, described by Tasaki *et al.* (31) while recording intracellularly from the visual cortex and interpreted as being related to a process of inhibitory hyperpolarization.

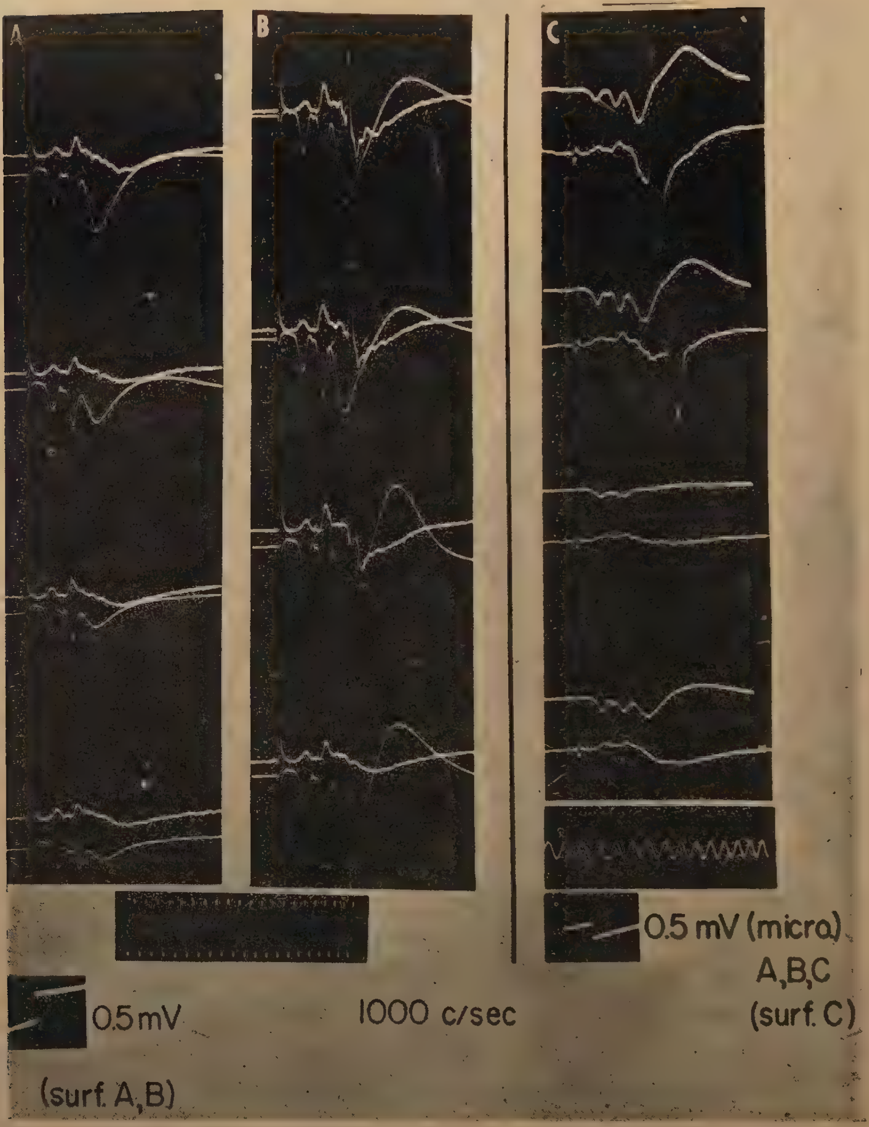

Fig. 10. – *Relationship between amplitude of surface response and unit discharge probability.*

Optic tract stimulation. *A*: Four consecutive responses of relatively small amplitude. *B*: Four consecutive responses of greater amplitude. Note presence of spike (related to wave 4) in three of them. *C*: Same phenomenon, different experiment. (Microelectrode tracing in upper channel; cortical response in lower channel). See also Figs. 11, 12.

5. *Relationship between size of surface response and probability of unit discharge.* — When working on lightly anesthetized animals, and using critical stimulus strength, one commonly observes large spontaneous fluctuations in the size of the surface response. If any causality exists between unit discharges and waves of the surface response, the probability of unit discharge should increase when the waves are large. As illustrated in Fig. 10, this is actually the case. Four records of a consecutive series (A) show relatively small surface responses and no spikes. In the other four tracings (B), the surface responses are well developed and in three of them a spike, temporally related to wave 4, is present. In C, from another experiment, the same relationship is shown: when the surface response increases the spike appears. This correlation is not absolute (see B), but the mean values of the amplitude of the surface waves are generally

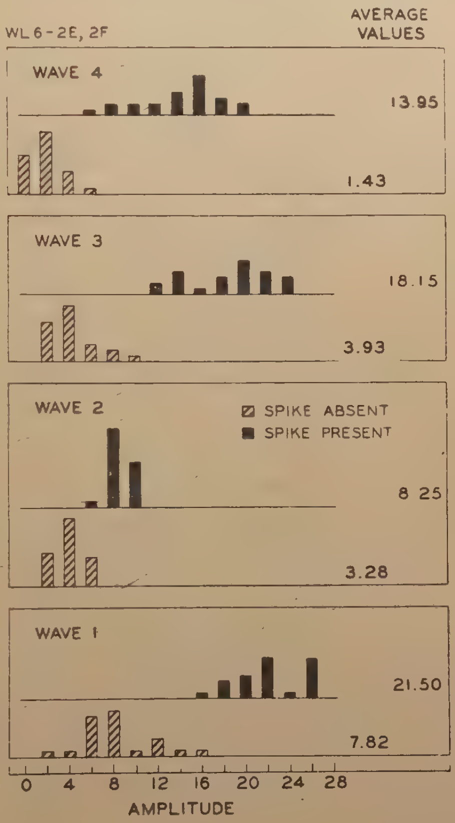

Fig. 11. – *Same phenomenon as in Fig. 10.*

Different experiment, optic tract stimulation. The amplitude of the first 4 waves of the same surface response (which showed no significant 5th deflection) have been plotted in two different situation: spikes present (black columns) and spike absent (striped columns). In the abscissa are the wave amplitudes (in arbitrary units) with the average amplitude values shown on the right side. The height of the columns corresponds to the number of observations for each value.

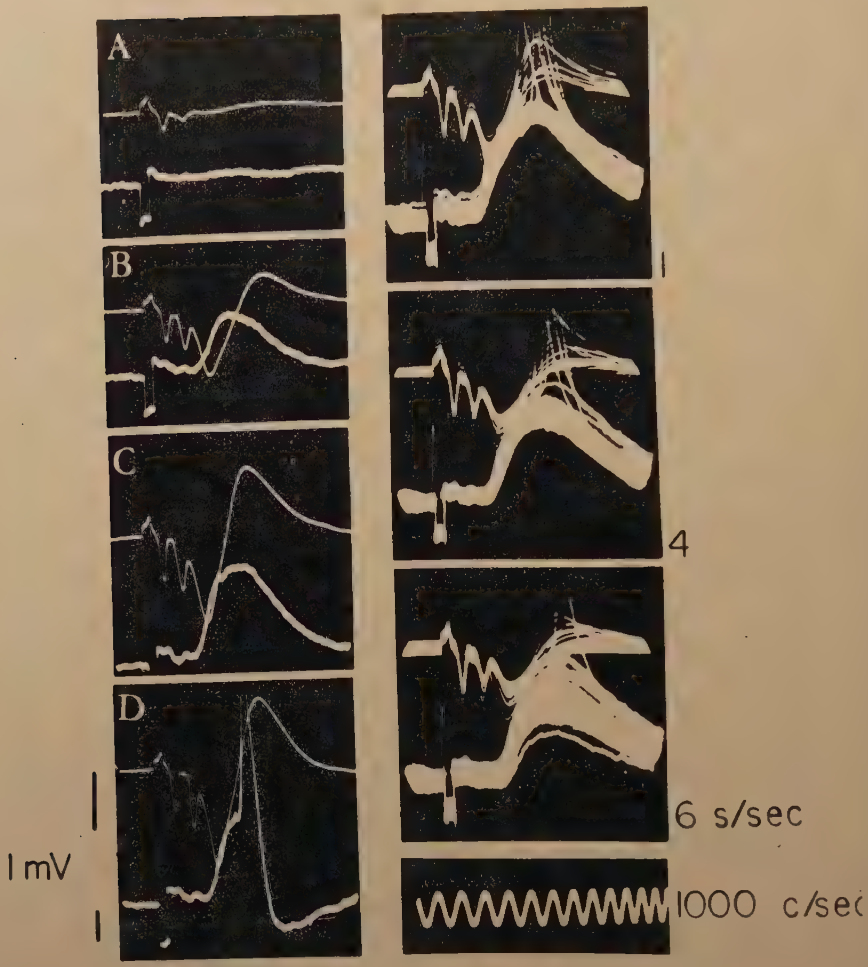

Fig. 12. — *Relationship between amplitude of surface response, and of slow pre-potential and spike discharge.*

A-D: Spontaneous variations of same response arranged in progressive order. Note spike discharge in *D*. Right column: Superimposed frames. Same response studied with repetitive stimulation at progressively increasing frequency, as indicated on right side of records. (Microelectrode tracing in lower channel; cortical response in upper channel).

and consistently higher when the spike is present than when absent. This is illustrated in the histograms of Fig. 11 obtained from another experiment: a trend for higher values when the spike is present is clear, and the greatest increase is noted for waves 3 and 4.

In the experiments of Figs. 10 and 11, the stimulus was applied to the optic tract and this explains the variations in amplitude also of the 1st and 2nd waves. When, instead, the stimulus is given to the optic radiation, there is hardly any appreciable fluctuations of these waves, and correspondingly, unit spikes recorded during the 1st and 2nd wave, as well as 3rd wave spikes of pre-synaptic type are very stable and usually respond to every optic radiation stimulus, once their threshold is reached (cf. Fig. 3, *a*-*e*).

The visual response to stimulation of the lateral geniculate body has been reported (12) to be greater in size during illumination than in the dark. We have also observed this phenomenon, but in our experience it is not constant (cf. 22): sometimes the response is increased, sometimes decreased by diffuse illumination of the retina and following optic radiation stimulation some units respond with higher, others with lower probability of discharge when the retina is illuminated.

The augmentation of surface waves is also related to presence and size of a slow prepotential recordable in favorable conditions and from which the spike potential arises when it reaches a certain size. The prepontential is a positively going graded response which could be considered analagous to the "first component" of Freygang (16)[1]. The spike does not always take off from the highest point of the prepotential, but sometimes late on its descending phase (phenomenon also observed and discussed by Freygang, 16). Fig. 12 (*A*-*C*) show concomitant changes in size of the slow prepotential and of the waves of the surface response. As shown in *D*, when both are maximal, the spike discharges. In the same figure the effect of increasing frequency of stimulation is shown: the surface response and particularly waves 4 and 5, progressively decrease together with the slow prepotential and the probability of spike discharge.

Discussion

Our criteria for the classification of the units as pre- or post-synaptic need some comments. In barbiturate preparations it has been found that in the thalamic or cortical sensory relay systems a strong conditioning shock is usually followed by a deep and long-lasting period of decreased excitability (21, 23, 29). Units in the somatosensory cortex were found to be completely unresponsive for 20-30 msec after conditioning stimulus when nembutal was used as anesthetic (25). If, therefore, the longlasting unresponsiveness in our experiments with " post-synaptic " units is not quite unexpected, one could challenge the conclusion that all units which do not show this kind of prolonged depression are necessarily " pre-synaptic ".

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[1] According to his analysis of extracellular potentials from single neurons in cat lateral geniculate nucleus, the "first component is dependent upon the synaptically initiated post-synaptic depolarization".

It may be possible that also in the visual cortex synapses exist similar to those described in the lateral geniculate nucleus (6) and characterized by very short absolute refractory period. Some changes in excitability with changes of the relative strength of conditioning and test shocks, however, would always be expected in a post-synaptic neuron pool, provided the relationship between pre- and post-synaptic units is not 1 to 1 (see 23).

The large afferent fibers to the visual cortex branch extensively in it and each thalamic fiber may directly influence the state of about 0.1 cubic mm of cortex in which lie 5000 neurons (30). There are, thus, abundant opportunities for overlap. The units classified as pre-synaptic in the present paper, however, were insensitive to changes in the strength of supraliminal conditioning and test shocks nor could any facilitatory phenomena be demonstrated with subliminal test shocks. These findings substantiate the assumption that these unit spikes are actually pre-synaptic, *i. e.*, impulses in afferent thalamo-cortical fibers.

In analogy it may be challenged that unresponsiveness during repetitive stimulation is a valid proof that the unit under study is " post-synaptic ". It could be a cell with a corticofugal axon antidromically stimulated. About 75% of the fibers at the boundary between visual cortex and white matter would be, according to Sholl (30), efferent and, if this is true, with radiation stimulation the chances of picking up antidromic impulses would be great. In Phillips' experiments (27), some Betz cells fail to follow antidromic stimulation at a frequency of " 50 c/sec or less ", yet, when tested with paired antidromic stimuli, these cells are able to respond to a test shock only 0.5 msec after the conditioning one. This was never noted in our " post-synaptic " neurons and, in addition, the Betz cells do not show the variability in latency and in probability of discharge which is instead characteristic of the post-synaptic units of the visual cortex under certain experimental conditions. It is, therefore, unlikely that a significant proportion of units labeled by us as post-synaptic be antidromically activated cells.

The primary aim of this investigation was to study the relationship between the various phases of the visual response recorded from the surface of the cortex and the unit activity in the cortex and underlying white matter. Consequently, only units discharging during the course of the surface response (definitely the large majority of all those recorded), have been considered and analyzed. It is evident that our method of relating a unit to a particular wave of the surface response is somewhat arbitrary, and that with another way of measuring onset and duration of waves a slightly different distribution of units might have been obtained. On the other hand the exact boundaries of the waves are difficult to establish, particularly in the case of 2nd and 3rd waves, and in some borderline cases the final decision is a matter of choice. The classification of units adopted, if not unobjectionable, is nevertheless operationally well defined and the significant and meaningful differences demonstrated between the various groups, indirectly suggests its validity.

The fact that a unit happens to discharge during the course of a particular wave of the surface response, does not, of course, permit the conclusion of a causality between the two events. There are, however, some hints that the relantionship is not merely a temporal one. As demonstrated in Fig. 5 (*F* to *I*) and Figs. 10, 11, and 12 there is a direct correlation between size of the surface waves on the one hand, and probability of spike discharge, as well as size of the slow pre-potential on the other hand. This is true with spontaneous fluctuations in size of the waves or with fluctuations induced by changes in the stimulus strength or by a preceding conditioning shock. The waves most affected by these fluctuations are the 4th and 5th ones, and spikes discharging in correspondence with these waves are those most variable and unstable. These spikes also show variations in latency, this being shorter, the larger the late surface waves (Fig. 10, *C*). As pointed out above, the 1st and 2nd waves are, instead, very stable, particularly when evoked by optic radiation stimuli, and so are the spikes related to them (Fig. 3). The 3rd wave is more variable than the two first, but much less so than the late waves, and spikes related to it have been shown to be of both presynaptic stable type, and post-synaptic variable type.

Also the topographical relationships demonstrated in Fig. 2 suggest more than a mere temporal coincidence between waves and unit action potentials. During the 1st wave, which is unanimously considered to represent action potentials of radiation fibers, the unit discharges are picked up almost exclusively in the white substance. As the primary thalamic afferents end in layer IV (26) or III (30), one would have expected also intracortical 1st wave spikes. The reason for their scantiness is not clear. It may be due to their dichotomizing repeatedly within the cortex, the branches being so small that either with our technique we are unable to record their action potentials, or the conduction velocity decreases abruptly so that the spikes are delayed and recorded with relation to the 2nd or 3rd waves. During the 3rd wave, units are recorded both in the deepest and most superficial layers explored, some behaving like radiation spikes, others like post-synaptic units. These findings may explain the great divergencies of opinion on the nature of this wave. Some authors state that it disappears after cortical ablation (3, 4, 7, 8, 22). Others (11-14) that it remains. According to Chang and Kaada (14), it is very resistant when the cortex is exposed to mechanical pressure or to the action of strychnine or local anesthetics. Malis and Kruger (22) state, on the other hand, that it is very sensitive to local procaine (though, judging from their Fig. 4, it is much less sensitive than waves 4 and 5). The presence of both pre--and post-synaptic elements in the 3rd wave may explain why some authors (3, 4, 24, 28, 29) have considered it of post-synaptic nature whereas, others (7, 8, 14) consider it to be a pre-synaptic response.

Comparatively few spikes have been picked up during the course of the small 2nd wave, and these are distributed between cortex and white matter. While this may explain why the 2nd wave has been variously described as due to activity in intracortical structures (3, 4, 7, 8, 24, 28) and in optic radiation fibers (14, 22), our findings offer no explanation for the differences in opinion with respect to its character of pre- or post-synaptic response, as we have found only elements suggestive of pre-synaptic type corresponding to this wave.

As demonstrated by Fig. 2 there is a trend for the late spikes to be picked up in more superficial layers and the few units discharging during the initial phase of the 5th wave are almost exclusively found in the cortex. Within the cortex no clear trend in the spatio-temporal distribution of units is apparent. The almost complete lack of discharging units during the middle and late parts of the large surface negative 5th wave, described also by Grutzner *et al.* (18), is surprising. Various explanations are possible. We only exceptionally recorded unit activity in the most superficial cortical layers above the level of about 500 μ. The 5th wave may be generated in superficial structures, *e. g.*, dendrites (cf. 3, 4) or small cells, whose activity is difficult or impossible to pick-up with our technique, or the tip of the microelectrode introduced through the pia, pushing the surface of the cortex downwards until it suddenly penetrates, may skip the most superficial layers. If the structures responsible for the 5th wave are situated in laminae I or II of the cortex, we would have no chances to record from them.

It has been suggested by Bishop and O'Leary (5) and Marshall *et al.* (24) that the negative 5th wave is due to activity in neurons whose axons leave the cortex. If this were true, one would expect to find some deep corticofugal discharges during the course of the 5th wave. This is, however, not the case and unit potentials picked up in the white substance are exclusively related to the early waves (Fig. 2). If some of them are corticofugal impulses, the reflex arc of the visual cortex activated in this way must be rather short.

Malis and Kruger (22) found two fibre groups of different conduction velocities in the optic radiation, one of 25-30 m/sec, the other of 9-11 m/sec. They consider them responsible for waves 1 and 2 of the surface response and state that the lantencies of the later deflections of the response, waves 3 to 5, are too long to correspond to any known conduction velocities of the optic tract. In the present work, no serious attempt was made to calculate the conduction velocity for impulses of pre-synaptic type. The distance between the stimulating electrodes close to the lateral geniculate nucleus and the recording microelectrode is about 15 mm. One of the slowest pre-synaptic spikes found is shown in Fig. 5. Its latency is between 1.5 and 1.75 msec indicating a conduction velocity of 8.5 to 10 m/sec. This value is close to the one given by Malis and Kruger (22) for the slowly conducting fibers of the optic radiation but the spike in question definitely corresponds to wave 3.

SUMMARY

1. A microeletrode investigation in cortex and underlying white matter of the visual area, following electrical stimulation of optic radiation, was carried out in lightly nembutalized cats.

2. The majority of spikes recorded discharge during the course of the specific evoked surface response and may be temporally related to its various components.

3. On the basis of latency and of their behaviour when tested with double shocks and repetitive stimulation, all spikes may be divided into two groups: "pre-synaptic" and "post-synaptic".

4. All spikes temporally related to the first wave and most spikes firing during wave 2 are recorded from white matter and all have pre-synaptic characteristics. Those temporally related to wave 3 have the most extensive spatial distribution being picked up in both cortex and white matter: some behave as pre-synaptic, others as post-synaptic events. The spikes related to wave 4 and the rare ones related to wave 5 are all of a post-synaptic type and most of them are only found within the cortex.

5. A positive correlation exists between presence of spike and amplitude of the surface response. This can be expressed as follows: first or second wave spikes appear when waves 1 or 2 have reached a critical size. The spikes then remain stable responding to every stimulus. A third wave spike of pre-synaptic type behaves similarly. Spikes of post-synaptic type related to waves 3, 4 or 5 also may appear when the first wave has reached a certain size; above this threshold value, however, they are "unstable", responding irregularly to stimuli of constant strength. Their probability of discharge is greater the larger the amplitude of the late surface waves.

6. These findings provide further confirmation of some of the current concepts on the nature of the various components of the "visual" response. They also offer an explanation of the difference of opinion still existing in regard to the nature of wave 3, which is considered as pre-synaptic by some and post-synaptic by other investigators. On the basis of our results, both pre- and post-synaptic elements are present in wave 3.

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EEG SYNCHRONIZATION ELICITED BY LIGHT [1]

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INTRODUCTION

Previous investigations have shown that darkness and visual deafferentation exert opposite effects on the EEG of the midpontine pretrigeminal cat (14, 1, 2). We found that the low voltage fast activity of the dark adapted animal was reversibly replaced by synchronous EEG patterns, with spindle bursts, during functional visual deafferentation (1, 2), a finding that was explained as a consequence of the ischemic abolition of the retinal dark discharge (2). The mechanisms by which light, dark and visual deafferentation effect the amplitude of the evoked potentials in the visual system was then the object of a second group of investigations (3, 5). Here again visual deafferentation and darkness were found to exert opposite effects, since the enhancement of the visual potentials occurring during continuous illumination of the retina (13, 21) was observed also as a consequence of the ischemic inactivation of the eyes (3, 5), an observation that was also attributed to the abolition of the dark discharge. The main conclusion was that darkness and visual deafferentation play an opposite rôle in the control of the EEG rhythms and of geniculate excitability in the midpontine pretrigeminal preparation (1, 2, 5). Although these observations hardly could be reconciled with the old assumption that absence of photic stimuli corresponds to visual deafferentation, they might pave the way to a better understanding of the functional significance of the retinal dark discharge.

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Continuous illumination and visual deafferentation had been shown to similarly affect the evoked responses of the visual system. Yet it might appear as a real gamble to start an investigation with the aim to find out whether EEG synchronization could be produced, in the midpontine pretrigeminal preparation, by continuous retinal illumination. No matter how hard it had been to conceive that suppression of all kinds of photic stimuli could be an important factor in the maintenance of EEG arousal patterns, still more difficult was it to believe that light should mimick visual deafferentation, thereby inducing synchronization of the EEG. This was, however, the result of our work. In fact *the present experiments show that for given geometrical and physical parameters of the photic stimulus, the EEG of the midpontine pretrigeminal cat may be synchronized by steady retinal illumination.*

METHODS

The experiments were carried out on cats prepared with midpontine pretrigeminal interruption of the brain stem. The technique for performing such a section has been previously described. (2)

The electrical activity was recorded unipolarly from the cortical surface, through the intact dura. The electrodes were silver-silver chloride and the contact was made through cotton wicks soaked in Ringer solution. A reference electrode built in the same way was imbedded in a large cotton pad lining the skull.

Recording was commonly made on a Grass ink-writing Polygraph, with 5P1 preamplifiers. Although the frequency range was limited on the high side (45 Hz), this apparatus proved to be very useful because it permitted the switching from A. C. to D. C. recording. In other instances D. C. and A. C. preamplifiers connected to a CRO system (Toennies) were utilized. In this case the linearity of the filters extended until 5 KHz. For D. C. recording suitable electrodes were utilized (6).

A common microscopy lamp was used as light source for retinal stimulation. The geometrical and physical parameters of the stimulus will be described in the text, as they represent rather critical factors in the appearance of the phenomenon. Since the experiments required illumination of the eyes from above, the skin and the superior lids were removed until the superior border of the orbit was exposed.

The technique for obtaining the reversible visual deafferentation through retinal ischemia has been reported in a previous paper (2; see 12). Because of the pretrigeminal level of the section, experimental procedures leading to strong nociceptive stimulation (extirpation of the upper lid, intraocular compression) were painless in our animal.

The animals were immobilized with Sincurarina [1] and artificially ventilated. Control experiments without the drug were also performed. As reported below, particular attention was devoted to the maintenance of optimum respiratory and circulatory conditions. To this purpose we ran routinely a series of controls: *i*) a double photocell oxymeter applied to the tongue was utilized to check the level of oxygenation of blood hemoglobin. The output from the photocells was fed to a Leeds and Northrup Speedomax millivoltmeter recorder through a cathode follower stage and calibrations made on samples of arterial blood, in which the oxygen content was measured with the spectrophotometric method of Gordy and Drabkin (16); *ii*) blood pressure was routinely controlled directly with an Hg manometer through a canula introduced into the femoral artery; *iii*) changes of cortical temperature, as index of circulatory conditions of the cortex, were recorded by thermistors connected to a bridge network and fed to one channel of the Polygraph; *iv*) finally, EKG was also regularly recorded.

RESULTS

Since the main object of our work was to analyse the experimental conditions that led to clear-cut change of the EEG we decided to concentrate our attention on those cases in which low voltage fast activity was replaced by typical synchronous rhythms and viceversa. We regarded as typical EEG manifestations of sleep only those EEG records in which we observed regular trains of spindle waves with slow activity in the interspindle lulls. Other EEG patterns, *e. g.*, slow rhythms without spindle trains or the appearance of a few occasional spindles on an activated background were taken only as indication that a change had supervened in a direction which might eventually result in a more clear-cut EEG sleep pattern if the experimental conditions could be properly adjusted. These records were occasionally of invaluable help to decide the direction that had to be given to the experiment, but they were not considered in the present work and will not be reported in the illustrations. We realize that the appearance or the disappearance of the spindles is by no means the unique sign of changes in the " central tone " of the cerebrum. In the absence of an EEG analyser, however, we thought it advisable to turn our attention to the best known and most striking among the EEG changes.

Paragraphs 1 to 4 will be devoted to a description of the results we obtained in those instances in which all conditions were at their optimum. We also wish to stress that our description applies *only* to those optimum conditions. An analysis of the most important factors involved will follow in the paragraphs 5 to 7. We hope it will give a fairly accurate picture of the limits within which our description holds true.

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[1] We wish to thank the Farmitalia (Milano) for kindly supplying the product.

1. *Effects of steady light.* — We have already seen that the low voltage fast activity of the dark adapted, acute midpontine preparation depends upon a steady flow of retinofugal impulses, the dark discharge (1, 2). The opposite condition, steady retinal illumination, is by itself a sufficient factor for inducing EEG synchronization, provided the photic stimulus is applied following geometrical and physical parameters to be specified in detail below (see paragraph 6).

These effects of retinal illumination arise very slowly, since they appear only after several minutes. The only immediate effects of light are represented 1) by the well know potential changes constituting the primary cortical response, which can be led from the visual areas at the onset of the illumination, and 2) by the longer-lasting (1 to 3 sec) slow potential oscillation (Fig. 1, *A*) that follows the primary response (6). Following these " on " effects a slow process seems to take place, whose occurrence, however, is only inferred, since no sign of it can be detected by simple inspection of the EEG recordings until a few minutes have elapsed. During this " build-up " time the light must remain on, that is, a steady stimulus is required. After a time interval from a minimum of 30 seconds to 1 or 2 minutes, one perceives in the records from the visual areas that slower and higher voltage rhythms are intermingled with the fast low-voltage waves (Fig. 1, *B*). These slow rhythms become gradually more evident until, usually within 5 minutes clear-cut EEG synchronization is present (Fig. 1, *C*, *D*). As already reported (2) the EEG synchronization is characterized in the visual areas by trains of waves at 4-9 peak per sec, only occasionally the repetition rate rising to 9-12 peak per sec. Although the synchronization clearly begins in the visual areas, it does not remain localized there. When the sequence of high voltage spindle trains is fully established in the records from the striate cortex, spindles will also appear in more frontal leads, showing the involvement of the whole neocortex. The waves of the spindle trains led from the frontal areas generally have a higher repetition rate, 9 to 16 peak per sec, but the onset of the trains usually coincides with those on the visual areas (Fig. 1, *D*). If the steady retinal illumination is maintained the sleep patterns persist and eventually become more clear-cut. Slow waves at 1-3 per sec appear occasionally in the record and the whole picture of synchronized activity may last for hours.

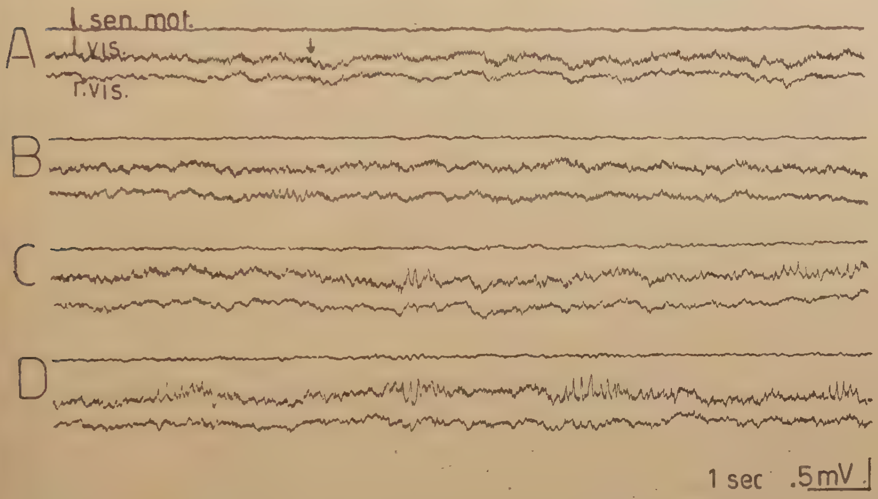

Fig. 1. – *EEG sleep induced by retinal illumination.*

C. R. O. recordings. Channel 1 through A. C. and Channel 2 and 3 through D. C. amplifiers. *A*: in darkness. The light at 90° angle is turned on at the arrow and is maintained throughout. *B*: 1 minute after the end of *A*. *C*: continuing from *B*. *D*: continuing from *C*. Slow trains appear first in the right cortex (*B*) but full sized spindles develop first in the left visual area (*C*). Beginning of synchronization of frontal area only in (*D*).

2. *Effects of olfactory stimuli on the EEG synchronization elicited by light.* — The low voltage slow waves produced by steady retinal illumination are readily disrupted by olfactory stimuli. A stream of room air introduced into the nostrils, even if not reinforced by odors, will abolish EEG synchronization, the spindle trains being suddenly replaced by low voltage fast waves. In the pretrigeminal preparation this effect is exclusively due to stimulation of the olfactory receptors. A remarkable feature of olfactory stimulation in these experimental conditions is represented by the long duration of the EEG arousal following a relatively brief period of olfactory stimulation; usually the arousal lasts not less than 2 minutes (Fig. 2) and frequently 5 minutes or more. If steady light is maintained, the arousal patterns give way once more to synchronous rhythms.

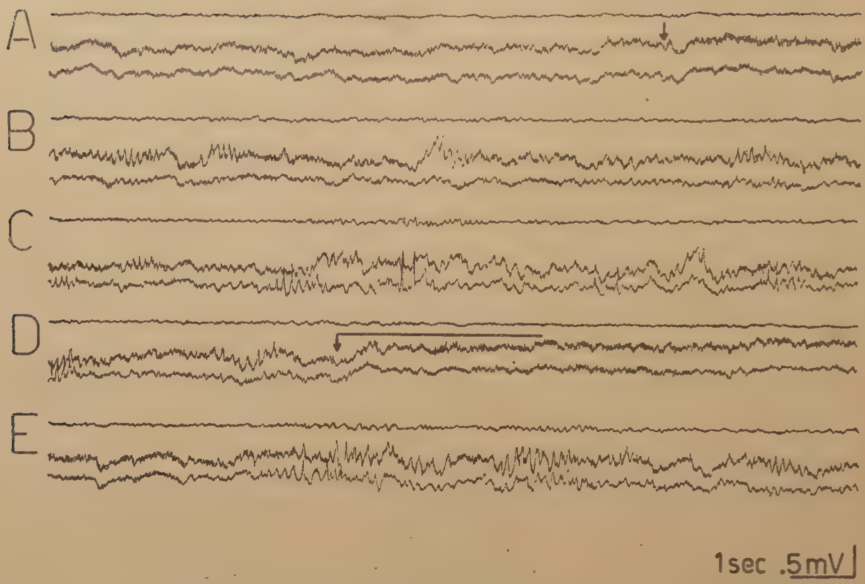

Fig. 2. – *Blockade of EEG synchronization elicited by light during sensory arousal.*

Same experiment of Fig. 1. C. R. O., recording from the same areas. *A*: darkness. The light at 90° angle is turned on at the arrow, and is maintained throughout. *B*: 2 minutes after *A*. *C*: continuing from B. *D*: 1 minute later. Olfactory stimulation (marked by the arrow) induces arousal and slow negative potential shift on the visual areas (D. C. recordings). *E*: 2 minutes later. In the middle of the record the first spindle train appear.

3. *Effects of darkness.* — When the light is turned off and the retinal receptors are left in complete darkness no sudden change can be detected at first, besides the evoked " off " potential on the visual area. Synchronized patterns do not disappear at once. On the contrary, at the very beginning of the dark period the spindle trains are sometimes more frequent and of higher voltage. After some time, however, the sleep picture seems to fade away, the interval between spindles lengthens and higher-frequency and lower-voltage activity occupies the interspindle lulls. The voltage of spindles may also decrease, until only slow rhythms of low amplitude remain They eventually disappear after a short time, leaving the pure patterns of low voltage fast activity. All these changes take place rather slowly, the most usual figures being around 10 minutes for complete disappearance of every sign of previous synchronization. Occasionally, the spindle trains suddenly disappear soon after the

onset of darkness or shortly thereafter, when their repetition rate had only slightly decreased. It would then be really impossible to differentiate the effect of withdrawal of steady illumination from the usual arousal reaction induced by sensory stimulation.

Low voltage fast activity invades the cerebral cortex in a reversed sequence as compared to EEG synchronization. Slow waves disappear first from the frontal areas and later on from the visual cortex. Hence, during the progress of both synchronization and desynchronization (usually for a longer period of time in the last process) the EEG activity is different in the frontal and in the occipital areas.

4. *Repetition of light and dark periods.* — When the process described in the last paragraph is completed low voltage fast activity may persist unmodified for quite a long time, if darkness is main-

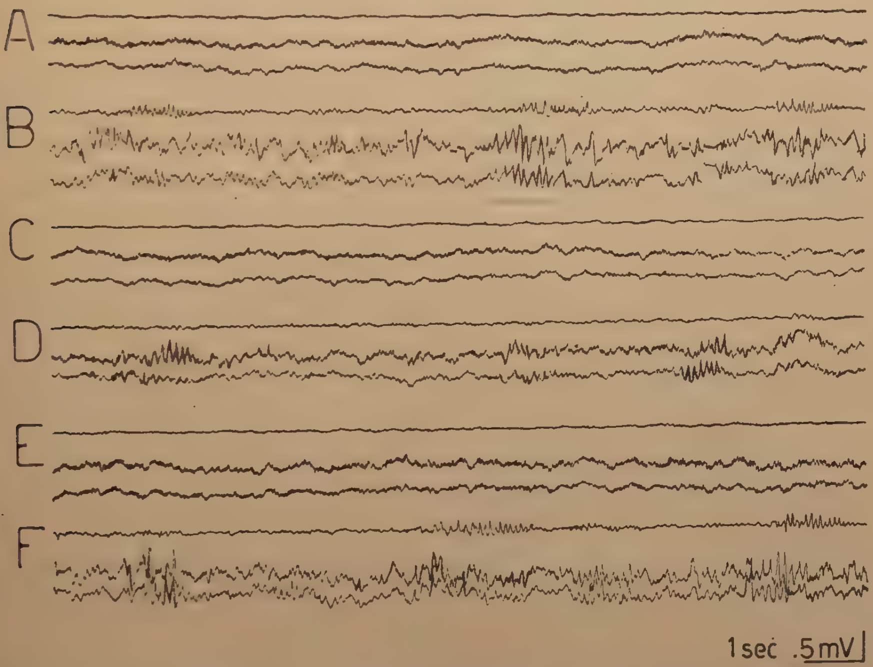

Fig. 3. – *Alternation of EEG synchronization and of low voltage fast activity induced by light and darkness.*

Same experiment of Fig. 1. C. R. O. recordings from the same areas. *A*: in darkness. *B*: after 2 minutes of illumination with light at 90° angle. *C*: after 5 minutes of darkness. *D*: after 1 minute of illumination with light at 90° angle. *E*: after 6 minutes of darkness. *F*: after 2 minutes in light at 90° angle.

tained. The preparation, however, repeats the already described EEG changes if light is turned on. We have been able, actually, to reproduce several times the reversible patterns of EEG synchronization and of low voltage fast activity simply by alternating for adequate periods of time steady illumination and darkness (Fig. 3). We have been unable to see clear-cut signs either of facilitation or of fatigue in these experiments. It is true that in several cases the reversibility of the EEG effects became more and more sluggish and incomplete, as time went on, but in other instances the latency of the phenomena actually became shorter and shorter and the EEG changes became clearer. The difficulties encountered in maintaining a preparation in stedy conditions for long time do not permit us to commit ourselves on the mechanisms of these differences of behavior.

5. *Effects of different levels of transection of the brain stem.* — As previously reported (2) in our experiments the pons was sectioned through a ventral approach with a thin spatule, a technique that minimized the injury to other parts of the nervous system, but one that permitted slight changes in the level of the section. This level was routinely controlled on serial histological slides, and we found that good effects were obtained in all instances in which the transection was made just in front of the trigeminal roots. The true level of the lesion could be traced, occasionally, more rostrally due to hemorrage developed at the time of the transection. However, a lesion at a slightly higher level did not jeopardize our results unless it extended near the rostral border of the pons. In these latter cases the EEG activity always showed the everlasting sleep patterns of the prepontine preparation (8, 9) and of the *cerveau isolé*. Darkness did not disrupt the synchronous rhythms in these experimental conditions, while short-lasting arousal was produced by olfactory stimulation, in confirmation of previous observations on the *cerveau isolé* cat (7). When the lesion was placed below the midpontine level, just sparing the trigeminal roots, or still lower or whenever it happened to be incomplete, sparing *e. g.* the auditory pathways, the behavior of the preparation was quite unpredictable. Periods of sleep and arousal followed each other independently of darkness and of illumination.

6. *Parameters of light stimulation.* — A critical factor in our experiments is represented by the geometrical and physical parameters of the photic stimulus. The importance of the retinal

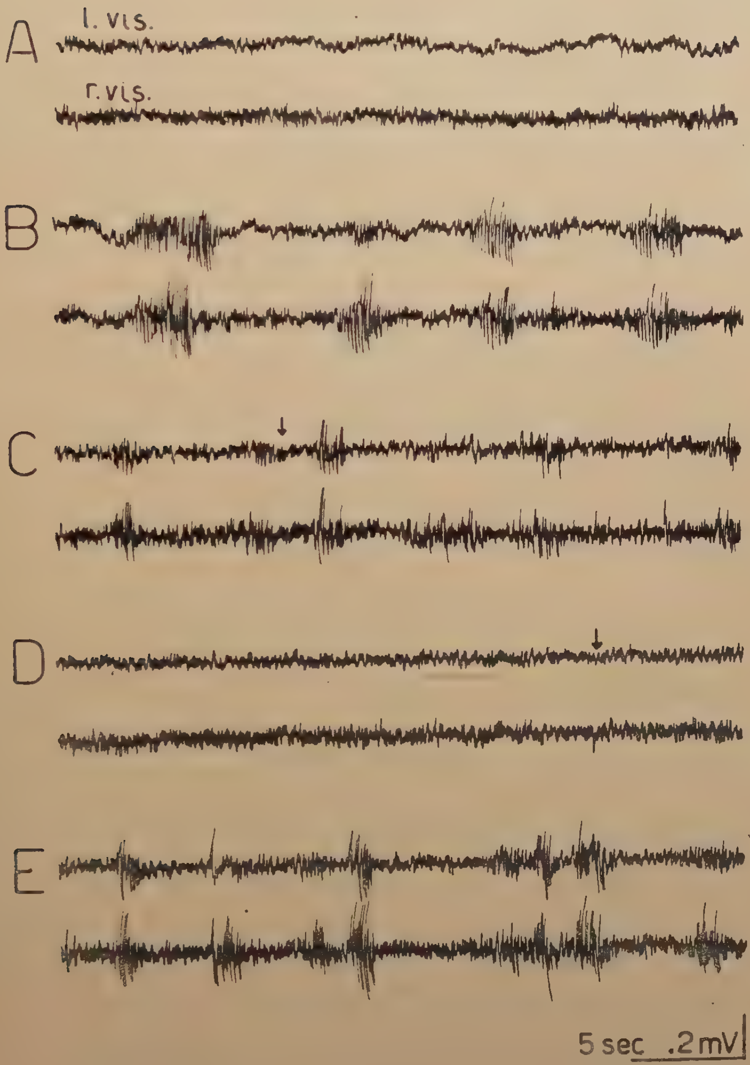

Fig. 4. – *Influence of the angle of incidence of the light beam on the light-induced sleep.*

EEG recording through *dura mater*. *A*: after 10 minutes of dark adaptation. *B*: 3 minutes after beginning of illumination with light at 90° angle. *C*: following *B*. At the arrow the light beam is brought parallel to the optic axis. *D*: 2 minutes after *B*. At the arrow the light beam is returned to a 90° angle. *E*: 3 minutes after *D*. Note that in *C* changing the direction of the light beam does not produce an arousal reaction.

illumination became apparent only when we realized that a series of unsuccessful experiments was due to changes in our technique of stimulation which we regarded at first as of minor importance An attempt was then made to analyse more closely these stimulation parameters. So far, only the effects of the direction of the light beam falling on the cornea and, to some extent, that of light intensity, have been investigated. More experiments are needed with controlled intensity and wavelength of the light stimulus.

Our first positive results had been obtained, as chance observation, with light falling from the ceiling. Since it was impossible to give in this way the intensity of the light falling upon the retina, we decided to utilize a light beam lying in the optic axis of the eye. To our surprise these experiments were completely negative. No matter how bright or how dim the light beam was, we found it altogether impossible to replace the low voltage fast activity of our midpontine cats with synchronous rhythms. Only when the light beam fell on the corneas from above, with an inclination of 45° to 90° to the horizontal axis, were we able to reproduce our effect.

Once the synchronizing effect of light falling with a 90° angle has been established it can be abolished by bringing the light beam in the optic axis (Fig. 4). This effect does not follow immediately the change of direction of the light beam (Fig. 4, *C*) as it would do if it was a kind of arousal reaction induced by the quick change of the geometry of stimulation. The EEG arousal patterns appear in a time comparable to that required by darkness to induce the same effects. At angles of incidence near 45° the changes of the EEG patterns, eventually leading to widespread synchronization all over the neocortex, generally appeared after a longer period of time than for larger angles; 10 minutes, more commonly 15 to 20 minutes of steady illumination were required to produce synchronization of cortical rhythms.

Our data on the intensity of photic stimulation are less clear. We have commonly utilized a Tungsten filament lamp carrying a current of 5 *A*, heated with 6 *V*, at a distance of 40 cm. from the corneal surface. The beam was concentrated over a circular area of 7 cm. in diameter at the level of the eyes, in order to ensure identical stimulation of both retinae. However, owing to the lack of suitable measuring device, we cannot give the absolute intensities of the stimulus we utilized. From a few rough measurements, obtained with a photocell, it seems that any increase in the intensity of light stimulation above the level utilized in our routine procedure makes it progressively more difficult to obtain the effect.

7. *Respiratory and circulatory factors.* — These two factors cannot be easily separated, since both contribute to the maintenance of optimum levels of pO_2 and pCO_2 in the internal environment of the brain. Two factors were controlled in our experiments, viz., blood oxygenation and blood pressure.

The EEG effects of steady retinal illumination were no longer observed when the percentage of O_2 saturation of Hb fell slightly below the maximum values. The opposite danger of hyperventilation leading to hypocapnia was avoided by adjusting the respiratory pump in such a way to maintain adequate O_2 saturation with the slowest rate and with the lowest depth of the respiratory acts. We found that a rate of about 18 strokes per minute was adequate for our immobilized animals; it is considerably lower than the rate of spontaneous respiration in the intact cat.

The limits of systemic blood pressure values were more easily defined. No experiment was performed when arterial pressure was less than 85 mm Hg. In the course of several good experiments an occasional circulatory collapse showed clearly that no effect was to be expected from light stimulation when the pressure was lower than this critical level. During circulatory collapse the EEG showed slow waves grouped in spindles and very little activity in the inter-spindle lulls even during dark adaptation. Only after injection of Ephedrine the low voltage fast activity returned, and occasionally those patterns were replaced by synchronous rhythms during retinal steady illumination.

Discussion

Our discussion will be centered around the neural mechanisms and the physiological significance of the EEG synchronization produced by steady retinal illumination, the EEG synchronization being regarded as the result of partial functional deafferentation of the visual inflow. An attempt will first be made at an evaluation of some objections which may be raised against our interpretation of the phenomenon. We shall try then to outline a working hypothesis based on the reduction of the dark discharge produced by visual illumination. The third part of our endeavour will be devoted to a critical appraisal of the limitations inherent in our experimental approach.

1. *A discussion on possible causes of error.* — Tension of blood gases is a factor that must particularly be taken into account in the EEG experiments performed under artificial respiration. In our curarized pretrigeminal cats the main cause of error is represented by an excess of ventilation. Let us assume that a slight hyperventilation leads to EEG synchronization. If this tendency is not too strong, it might be checked by the activating influence of the retinal dark discharge. The abolition of this discharge by ocular ischemia (12, 1, 2) or during steady illumination (as in these experiments) would restore the background of synchronization. If this hypothesis is correct it would be unjustified to state that steady light produces synchronization. These EEG patterns would be the consequence of an abnormality of the internal environment, namely of hypocapnia, and the light would simply uncover this effect. It is true that Batini, Magni, Palestini, Rossi and Zanchetti (8) have shown that the low voltage fast activity of the midpontine pretrigeminal preparation is not due to abnormal humoral factors, however their experiments were made in the chronic, non curarized, spontaneously breathing preparation, so that their results do not provide direct evidence against this objection. We regard it unlikely that our experimental results are due to hypocapnia for three reasons:

1) First of all, the synchronizing effect of light was obtained also when the usual intensity of artificial ventilation was somewhat reduced; it actually disappeared only if the percentage oxygen saturation of the arterial blood fell below a critical value. Although oxymeter reading obviously lack the precision of more quantitative blood gas measurements, there is little doubt that pO_2 was lower and pCO_2 actually higher than in the normal cat for the lowest intensities of ventilation that still permitted an EEG synchronizing effect of light to appear. Hence the synchronizing effect of light can be obtained also without hyperventilation.

2) The very slow irregular waves (1 to 3/sec) frequently observed during hyperventilation (15, 11) cannot be mistaken for the spindle trains. It is unlikely, moreover, that prolonged hyperventilation would permit us to obtain EEG activation several times, as we routinely evoked by means of olfactory stimulation.

3) It is not clear in terms of the hypothesis under consideration why the build-up time of the EEG synchronization produced by light should be so long (of the order of a few minutes). If the dark discharge would conceal an inherent tendency to synchronization produced by hypocapnia, the withdrawal of the activating discharge should be followed immediately by the synchronization.

It is easier to handle the opposite objection that the low voltage fast activity of our dark adapted preparation might be due to decrease of pO_2 and increase of pCO_2 produced either by hypoventilation or by decreased blood circulation in the brain. Effects of this kind, if present, should be quite slight, since blood oxygenation, arterial pressure and pupil diameter were routinely controlled in our experiments. Moreover, the initial EEG arousal produced by anoxia is due to chemoceptive discharge (15) which obviously cannot reach the cerebrum following a pretrigeminal transection.

Summing up, we believe that low voltage fast waves represent the standard EEG activity in our experimental condition, for normal levels of blood gas tension. Hence our problem is to analyse the neural events leading to EEG synchronization during continuous, prolonged illumination of the retina.

2. *A hypothesis on the EEG synchronizing influence of steady light.* — It is evident that the temporal and spatial organization of the impulse traffic along the optic nerve greatly differs in the dark adapted preparation from that of the same animal under steady light. Moreover, some of these differences must ultimately be responsible for the tendency toward EEG synchronization observed under steady light. Unfortunately our knowledge on the retinofugal discharges is quite fragmentary. Most of the authors were concerned with the events occurring at the onset and at the end of the retinal illumination, rather than with those elicited by steady light.

The most direct approach to a study of the retinal influences on the central nervous system is provided by microelectrode recording from single cells of the ganglion layer (17, 19, 18) or from single fibers in the optic nerve (12). The data obtained with this method (see 17, 18) are of invaluable usefulness in interpreting the events of retinal excitation, but there are two serious limitations in our knowledge which need to be stressed. First of all most of our information concerns the effects of short lasting photic stimulation recorded for a very limited stretch of time. Second, only a few samples of retino-fugal responses have been studied, and only one at a time.

Only recently an attempt has been made to investigate the behavior of the same retinal units during long-term stimulation, with the hope to offer a picture of the overall retinal activity in long stretches of time (20). The rather unexpected and striking result has been that most elements are active both in darkness and in light. Actually in most of the light-influenced elements the activity is stronger in darkness than during prolonged illumination. At the onset of illumination the rate of firing rises in the " on " and " on-off " elements (17, 19), but this level cannot be sustained for more than a few seconds in most of the units. If the photic stimulus lasts for some time, the activity of the " on " and " on-off " units falls below the firing level recorded during darkness. The dark discharge of other units (" off " type) is inhibited during retinal illumination (17). This inhibition cannot last as long as the photic stimulus (19), but the resumed discharge usually has a lower level than that in darkness (20).

Our EEG synchronizing effect might thus be due to the positive influence of photically evoked discharge or be the consequence of the withdrawal of a tonic influence arising in darkness. We believe that the second hypothesis is more likely, for the following reasons:

1) It is difficult to see how the " on " discharges of the retina may lead to EEG synchronization after several minutes from the beginning of the illumination. These " on " discharges are unlikely to be present in our experimental conditions in which the nystagmic movements of the eye were abolished by the curarization so that the intensity of the photic stimulation remained constant throughout the period of stimulation.

2) Visual deafferentation by ocular ischemia, performed in the same preparation, clearly reproduces the effects of steady retinal illumination both on spontaneous EEG activity (1, 2) and on the responses of the visual cortex to geniculate shocks (3, 5).

3) The enormous latencies of the EEG effects elicited by steady retinal illumination also support the idea that a slowly progressing process starts as soon as a critical decrease of the retinal dark discharge occurs. On the other hand, during the retinal blackout the suppression of the dark discharge is complete and the activating influence of light is altogether absent, two conditions explaining the earlier occurrence of EEG synchronization after ischemic deafferentation. According to our hypothesis *steady illumination would*

correspond to partial deafferentation in so far as the retinal dark discharge is concerned. The sudden withdrawal of the dark discharge would lead to a critical reduction of the tonic activity of the ascending reticular system at least in our experimental conditions, in which the sensory input is represented only by the olfactory and visual stimuli, thereby releasing the activity of the synchronizing pacemaker of the thalamus.

We realize that this is only suggestive evidence and that crucial proofs of our hypothesis are still lacking. However, the only alternative explanation of our findings would be to assume that besides the " on " and " on-off " units there are other retinal elements which are continuously discharging only under steady and prolonged illumination. This is an unproven and unnecessary hypothesis, which however cannot be dismissed in the present conditions of our knowledge. Our explanation, on the other hand, would fit Schubert's hypothesis (25) on the inhibition of the dark discharge as a signal of the intensity of steady illumination. The functional significance of the dark discharge was discussed in another paper (5)

3. *The limits of the deafferentiation hypotesis.*– We have already stressed, but it should be emphasized again here, that following midpontine pretrigeminal transection the effect of the withdrawal of the retinal dark discharge on the EEG rhythms is likely to be greatly enhanced by the absence of all other sensory inputs excepting the olfactory pathways. One might add that olfactory deafferentation alone is not followed by EEG synchronization in the midpontine cat (10). It is unlikely, moreover, that any unintentional stimulation of olfactory receptors by room air was present to a relevant degree in our animals, which breathed through the tracheal canula. In short, the striking EEG effects we observed during retinal black-out are likely to represent a somewhat distorted but greatly magnified picture of the tonic influence exerted by the visual input on the ascending reticular system. This influence is likely to be by far more subtle, and less easily detectable, in the animals with normal sensory connections. It is likely that the dark discharge is mainly concerned, at least in the cat, with the specific functions of the visual system, as shown by the fact that the EEG synchronization elicited by visual deafferentation (1, 2) and by steady illumination (this work) starts in the visual cortex even in our experimental conditions.

Even in the midpontine pretrigeminal preparation we have a conflict between the EEG synchronization elicited by withdrawal of the dark discharge and the activating influence of photically driven retinofugal discharges. The latter effect is actually likely to be overwhelming when repetitive flashes of light are applied, each of them giving rise to a pair of "on" and "off" discharges. In these experimental conditions EEG synchronization occurs only after several hours of repetitive stimulation (22, 23, 24) probably through mechanisms entirely different from those considered in the present work. But even with steady illumination the activating influences of light may easily override the slowly developing process of EEG synchronization.

Let us consider first the effects of varying the beam direction. EEG synchronization was obtained, in our conditions, only when the more peripheral receptors were illuminated. It is obvious that with an intact eyeball the receptors more centrally placed also receive a definite "amount" of stimulation. Our experiments should be repeated with more refined techniques of localized photic stimulation before any conclusion may be drawn regarding the EEG effects of the different parts of the retina. Our results may indicate as a working hypothesis that co-stimulation of centrally placed receptors should be limited as much as possible in order to obtain more easily EEG synchronization. As a matter of fact, all maneuvres leading to increased stimulation of centrally placed retinal receptors – such as reducing the angle made by the beam with the optic axis of the eye or increasing the intensity of light – prevented the appearance of EEG synchronization. Purely intraretinal interactions may play an important rôle in the struggle between the opposite effects of steady retinal illumination.

SUMMARY

1. Low voltage fast activity may last indefinitely in the dark adapted acute midpontine pretrigeminal cat.

2. Upon continuous illumination of the eyes there is a slow alteration of the EEG, stretching over a period of few minutes, until clear-cut patterns of EEG synchronization develop. The effect is obtainable only under certain conditions of the physical stimulus.

3. EEG synchronization reverts quickly to low voltage fast activity when olfactory stimuli are delivered (arousal reaction).

4. The spindle trains are replaced by low voltage fast activity when the illumination is withdrawn. This process also takes a few minutes to develop.

5. These alternations of opposite EEG patterns can be reproduced several times in the same preparation by alternating periods of steady illumination and of complete darkness.

6. The parameters of illumination and the general conditions of the preparation have been investigated.

7. The data are discussed and the results are compared with the effects of visual deafferentation.

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ELECTROPHYSIOLOGICAL ANALYSIS OF THE ACTION OF ATROPINE ON THE CENTRAL NERVOUS SYSTEM[1]

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INTRODUCTION

Electroencephalographic investigations on atropine were first carried out by Wescoe, Green, McNamara and Krop (23) and by Bremer and Chatonnet (8). These authors demonstrated that intravenous administration of the drug had a definite effect on the electrical activity of the cerebral cortex and, in particular, that it increased the amplitude and decreased the frequency of the electroencephalographic waves. This effect could not easily be reconciled with the results of behavioural experiments carried out a short time previously by Funderburk and Case (14), who had observed that the "avoidance" conditioned response of the cat was in no way modified by atropine. Further investigations by Funderburk and Case (15) confirmed the EEG effects of the drug and emphasised that the cortical electrical rhythms produced by it were identical with those observed during physiological sleep. In these early investigations, however, behavioural researches and electroencephalographic study had never been combined. Wikler (24), using intact dogs, neither curarised nor anaesthetised, was the first to study in the same animal the simultaneous effects of atropine on behaviour and on cortical electrical activity. In this way he was able to demonstrate that in the atropinised animal an EEG picture entirely similar to that of profound sleep was accompanied by alert behaviour.

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[1] English translation by T. D. M. Roberts and J. D. Christie, Glasgow.

On the basis of this " dissociation " between behaviour and EEG, Wikler maintained that " the mechanisms which subserve ' sleep ' and those which subserve the ' burst-slow ' wave EEG patterns are distinct from each other ".

The interest aroused by Wikler's observations, which were subsequently confirmed by Bradley and Elkes (4), is easily understood when one takes into account the importance commonly assigned to cerebral electrical activity as an index of behaviour, an index particularly valuable in those experimental conditions which preclude direct analysis of the behaviour itself. These observations have led to a large number of investigations into the mode of action of atropine on the central nervous system. Considering the well-known importance of the effect produced both on cerebral electrical activity and on behaviour by certain structures in the brain stem and diencephalon, it was natural that special attention should be paid to the nature of the effect of the drug on the activity of these structures.

By administering atropine to curarized rabbits, Rinaldi and Himwich (21) observed, according to the doses used, a rise in threshold or a block of the EEG arousal reaction in response not only to physiological stimuli but also to direct electrical stimulation of the reticular substance. They therefore maintained that the drug acted by depressing those subcortical structures which exert an " activating " effect on the EEG (the " mesodiencephalic system " in the authors' nomenclature). On the basis of this and of the fact that acetylcholine or other cholinergic substances, in contrast to atropine, produce a desynchronization of the EEG, they put forward the hypothesis that these structures have a basically cholinergic nature. Longo (17, 18) reached virtually the same conclusions at about the same time. He observed, also in the rabbit, a block or a depression of the EEG arousal reaction in response to physiological stimuli and to high-frequency electrical stimulation of the reticular substance of the brain stem and of the aspecific nuclei of the thalamus. Longo noted further that the behavioural responses to stimulation of the hypothalamus in intact, non curarized, rabbits were not affected by the drug.

Rinaldi and Himwich (22) maintained that support for the " mesodiencephalic " hypothesis of the site of action of atropine was provided by the fact that the drug has no effect on the cortical electrical activity of the " isolated hemisphere " preparation, a preparation in which the entire cortex of one hemisphere, with part of the basal ganglia, is deprived of all connection with the rest of the encephalon. We are of the opinion, however, that this conclusion rests on a rather weak experimental basis. We maintain that in the preparation of Rinaldi and Himwich, in which acute surgical section has separated one part of the telencephalon from the other parts of the central nervous system, the EEG activity is markedly depressed and uniform and does not lend itself to a confident interpretation of this sort.

By experimenting on the *encéphale isolé* cat, Bradley and Key (6, 7) demonstrated that, although atropine suppressed the effect of electrical stimulation of the reticular formation on the EEG rhythms, it did not impede the behavioural responses characteristic of this stimulation. Bradley and Elkes (5) further, confirming previous observations of Bradley, Cerquiglini and Elkes (3), and of Rinaldi and Himwich (22), observed that, in a *cerveau isolé* cat whose cerebral electrical activity had previously been desynchronized by the administration of a cholinergic drug, the injection of atropine restarted the profound EEG synchronization characteristic of the preparation. On the basis of these results, Bradley, Elkes and Key (5, 7, 2) did not consider it possible that the action of atropine on the EEG rhythms was due to a depressant action on the ascending reticular system. They thought that atropine acted on systems which are situated rostral to the reticular structures and which are not directly involved in the mechanisms that govern behaviour. As a hypothesis, they suggested that these systems might be found in the diffusely-projecting thalamic nuclei.

To sum up, it seems that there is general agreement in assigning a predominantly subcortical site to the action of atropine. Whereas for Rinaldi and Himwich (21, 22) and for Longo (17, 18) this site would cover the reticular structures of the brain stem and the aspecific thalamic nuclei, for Bradley, Elkes and Key (5, 7, 2), only the latter would be depressed by the drug.

In the present investigations we wished to re-examine the problem of the mode of action of atropine in inducing EEG synchronization and abolition of the arousal reaction. With this aim we have tried to carry out experiments to provide a direct indication of the action of atropine on the functional state of the reticular and thalamic structures that influence cerebral electrical activity and are responsible for the arousal reaction.

The results obtained from these experiments show that atropine acts at a supra-reticular level and suggest that the origin of the

EEG manifestations produced by the drug is profoundly different from the origin of those observed in barbituric sleep and in the *cerveau isolé* preparation.

METHODS

The experiments were carried out on 33 adult cats. The majority of the animals were subjected, under ether anaesthesia, to transverse section of the neuraxis with a spatula at the intercollicular midbrain level (*cerveau isolé* preparation) or at high spinal level, between the first and the second cervical segment (*encéphale isolé* preparation). Sometimes the spinal section was accompanied by electrocoagulation of both the Gasserian ganglia (the gasserectomized *encéphale isolé* preparation). In some animals, instead of complete surgical section of the neuraxis, a limited destruction of the midbrain tegmentum was carried out with a stereotaxic electrolytic technique. Some experiments were carried out on intact animals immobilized with Sincurarina and maintained under artificial respiration.

The cortical electrical activity was recorded on a 10-channel Galileo electroencephalograph using cotton wick electrodes placed on the sensorimotor cortical areas, exposed or protected by the *dura mater*, and screw electrodes fixed in the cranium in positions corresponding to the parietal and occipital areas. Bipolar concentric electrodes, inserted with the stereotaxic instrument, were used for recording from and for stimulation (always bipolar; square pulses of 1 msec duration) of the reticular and thalamic structures. Single electric stimuli applied to the sciatic nerve (1 msec) or single acoustic stimuli (produced by a loudspeaker connected to the stimulator) were used for the study of the reticular responses to peripheral sensory stimulation. In the experiments on the effects of atropine on the sensori-reticular and on the reticulo-reticular responses, the reticular potentials were observed and recorded on a Cossor oscillographic unit as well as on the electroencephalograph.

The atropine (sulphate) was administered intravenously or intraperitoneally in doses varying from 2 to 4 mg/kg. At the end of the experiment the animal's brain was perfused with 10% formalin by intracarotid injection and the electrodes were left *in situ* for 12 to 24 hours. The precise location of the electrodes was then determined by searching for their tracks in serial sections stained alternately according to the methods of Nissl and of Weil.

RESULTS

1. *The effects of atropine on the EEG arousal reaction to stimulation of the reticular substance and of the aspecific thalamic nuclei, and on the thalamo-thalamic and thalamo-cortical recruiting responses.* — At the beginning of the experiment we determined *a*) the lowest intensity of high-frequency stimulation (50-300/sec) of the reticular substance and of the aspecific nuclei of the thalamus which would produce complete desynchronization of the cerebral electrical rhythms (Fig. 1: *A*, 1 and 2), and *b*) the lowest intensity of low-frequency stimulation (6-12/sec) of the same thalamic nuclei which would induce recruiting responses either in the cerebral cortex (thalamo-

cortical recruitment) (Fig. 1: *A*, 3) or in the aspecific thalamic nuclei of the opposite side (thalamo-thalamic recruitment). Atropine was then injected in amounts varying from 2-4 mg/kg. After some minutes we observed the development of the well-known modifications of EEG activity, *i. e*, the appearance of high-voltage slow waves and of spindles at 8-12/sec in those animals in which the electrical activity was desynchronized, and an increase in amplitude

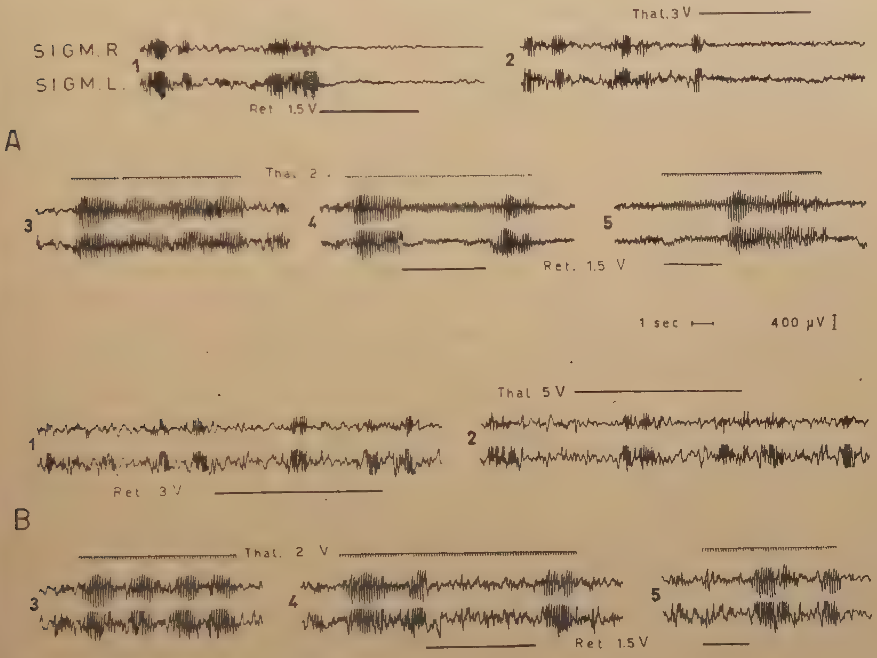

Fig. 1. – *Effects of atropine on the EEG arousal reaction, on the thalamically induced recruiting activity and on its reticular inhibition.*

Cat with partial destruction of the midbrain tegmentum. Electrocorticographic recording from the gyrus sigmoideus anterior of the right (*SIGM. R*) and of the left (*SIGM. L.*) side. Concentric stimulating electrodes in the left thalamic nucleus centralis medialis (*Thal.*) and in the midbrain reticular formation (*Ret.*).

A. Control. *1*: EEG arousal by reticular stimulation (1.5 V, 1 msec, 300/sec); *2*: EEG arousal by thalamic high-frequency stimulation (3 V, 1 msec, 100/sec). *3*: recruitment by low-frequency thalamic stimulation (2 V, 1 msec, 8/sec). *4* and *5*: strong depression of recruiting activity by reticular stimulation (1.5 V, 1 msec, 300/sec).

B. Fifteen minutes after the i. v. injection of 2 mg/kg of atropine. *1* and *2*: failure of reticular stimulation and thalamic high-frequency stimulation to produce EEG arousal. Note that the intensity of the stimulation is much higher than in A 1 and 2. *3*: recruitment by low-frequency thalamic stimulation as in A 3. *4* and *5*: reticular inhibition of the recruiting activity as in A 4 and 5.

of the slow potentials and of the spindles in those preparations which already had an EEG pattern corresponding to sleep. Physiological sensory stimuli no longer had any activating effect on the cerebral electrical rhythms. Similarly, high-frequency stimulation of the reticular substance and of the aspecific thalamic nuclei, with the lowest intensities which had been effective before the administration of the drug, no longer produced any modification of the EEG activity (21, 17, 18, 6, 7; Fig. 1: *B*, 1 and 2). Only when we used stimuli of higher intensity (equal to at least twice the previous threshold value) was it sometimes possible to bring about a disappearance of the spindles, but not of the slow waves, during the actual period of stimulation (17, 18).

In contrast to what was observed in the case of the EEG response to high-frequency stimulation, low-frequency stimulation of the diffusely-projecting thalamic nuclei still gave rise to recruiting responses in the cerebral cortex (17, 18; Fig. 1: *B*, 3) or in the contralateral thalamic nuclei, with no apparent change in the threshold.

2. *Effects of atropine on the reticular responses to single sensory stimuli.* — It is well known that single electrical stimuli applied to sensory nerve-fibres or natural stimuli of brief duration produce evoked potentials in the reticular substance, and that these responses are readily depressed by the administration of barbiturates (13, 1). The first step in our experiment was to locate a point in the reticular substance – usually at midbrain level – which responded well to electrical stimuli applied to the sciatic nerve and to acoustic stimuli, in each case the repetition rate being 0.5/sec. The latency, form and amplitude of the evoked reticular potential were determined. The second step was to determine the threshold of electrical stimulation at 300/sec which, when applied to this same point in the reticular substance, produced the typical EEG arousal reaction (Fig. 2: 1). Pentothal (Thiopentone sodium) was then administered intravenously (10-20 mg/kg). Its depressant action on the potential evoked by the sensory stimuli and on the EEG reaction to stimulation of the reticular substance provided us with a pharmacological control of the reticular nature of the phenomena observed (Fig. 2: 2). We then waited until, after the effect of the barbiturate had ceased, the evoked reticular potential reappeared and regained its original latency and amplitude, and until high-frequency stimulation of the

reticular substance once again produced activation of the EEG rhythms at the original threshold values (Fig. 2: 3). At this stage atropine was injected in sufficient quantity to produce a block of the EEG arousal reaction to reticular stimulation. Just as Longo and Silvestrini had observed in the rabbit after administration of scopolamine (19), so atropine had no effect on the activity evoked in the reticular substance by afferent stimuli: potentials absolutely equal to and sometimes even larger than those seen before the administration of the drug could still be recorded from the reticular substance in response to sciatic or acoustic stimulation (Fig. 2: 4).

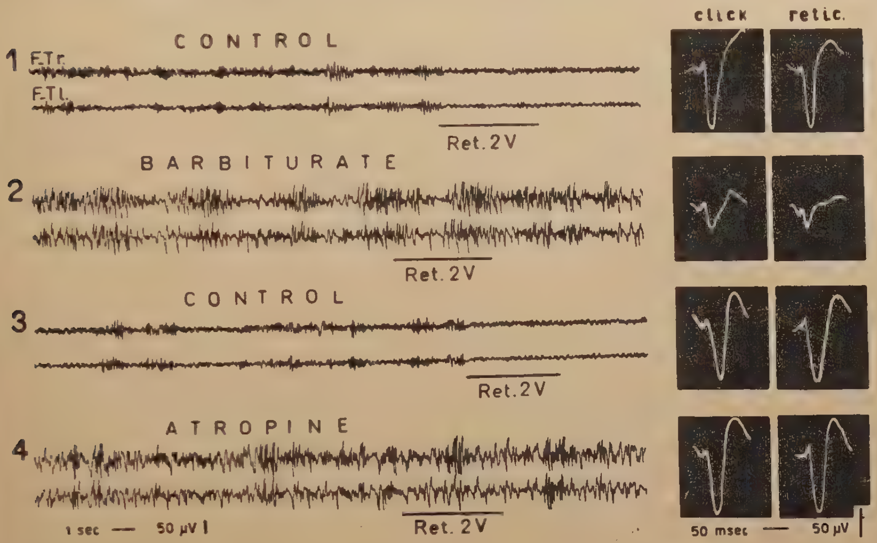

Fig. 2. – *Effects of Thiopental Sodium and of atropine on the EEG arousal reaction to reticular stimulation, on the reticular responses to single sensory stimuli and on the intrareticular conduction.*

Encéphale isolé cat. EEG recording from the fronto-temporal regions of both sides (*F. T. r.* and *F. T. l.*) and CRO recording from the mesencephalic reticular substance. *1.* Control: EEG arousal produced by electrical stimulation of a point of the midbrain reticular formation (2 V, 1 msec, 300/sec); responses evoked at the same reticular point by a single acoustic stimulus (*click*) and by a single electrical shock to the bulbar reticular substance (*retic*). *2.* Five minutes after the i. v. injection of 10 mg/kg of Thiopental Sodium: EEG synchronization, suppression of the EEG arousal reaction to reticular stimulation, and strong depression of the reticular potentials evoked by click and bulbo-reticular shock. *3.* Thirty minutes after 2: EEG arousal and evoked potentials as in 1 (recovery from barbiturate anesthesia). *4.* Ten minutes after the i. v. injection of 2 mg/kg of atropine: EEG synchronization and suppression of the EEG arousal reaction as in 2, but no changes on the amplitude of the reticular responses produced by click and bulbo-reticular shock.

3. *Effects of atropine on the mesencephalic reticular responses to single stimuli applied to the bulbo-pontine reticular substance.* — We began the experiment by locating a point in the reticular substance of the midbrain which gave good responses to the stimulation with single pulses, repeated every two seconds, of another point in the reticular substance of the pons or of the bulb. After the latency, form and amplitude of the response had been established, we determined the lowest intensity of stimulation at 300/sec of these same reticular regions which would produce activation of the electrical rhythms of the cortex (Fig. 2: 1). As in the experiments described earlier, we then went on to administer pentothal in order to check the sensitivity of the reticulo-reticular evoked potentials and of the EEG activation by reticular stimulation to a drug which unquestionably depresses the activity of the ascending reticular system: both effects were abolished by the barbiturate (Fig. 2: 2). When sufficient time had passed for all the effects of the pentothal to have disappeared (Fig. 2: 3), atropine was injected until the reticular activating effects on the EEG ceased completely. On examining the reticulo-reticular responses we noticed that they had not been in the least affected by the administration of the drug: the potentials evoked in the mesencephalic reticular substance by stimulation of the bulbo-pontine reticular substance had preserved their original latency, form and amplitude (Fig. 2: 4).

4. *Effects of atropine on the inhibition produced by stimulation of the reticular substance on thalamo-thalamic and thalamo-cortical recruitment.* — In these experiments, as in the others, we determined the threshold of reticular stimulation activating the EEG (Fig. 1: *A* 1; Fig. 3: *A*). We then sought to establish the lowest intensity needed for the same reticular stimulation to inhibit the recruiting responses induced by thalamic stimulation at 6-12/sec in the contralateral

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Fig. 3. – *Effects of atropine on the EEG arousal, on the thalamically induced recruiting activity and on its reticular inhibition.*

Cat with partial destruction of the midbrain tegmentum. EEG recording from the frontal (*F.*) and temporal (*T.*) regions of both sides (*R.* and *L.*). Concentric stimulating electrodes in the right thalamic nucleus centralis medialis (*THAL.*) and in the mesencephalic reticular formation (*RET.*). *A*, *B*, *C*: control. *D*, *E*, *F*. 20 minutes after the i. v. injection of 2.5 mg/kg of atropine. *A* and *D*: effect of reticular excitation (1 msec, 300/sec). *B* and *E*: effect of thalamic low-frequency stimulation (1 msec, 8/sec). *C* and *F*: effect of reticular stimulation (1 msec, 300/sec) on the thalamically induced recruiting activity (1 msec, 8/sec).

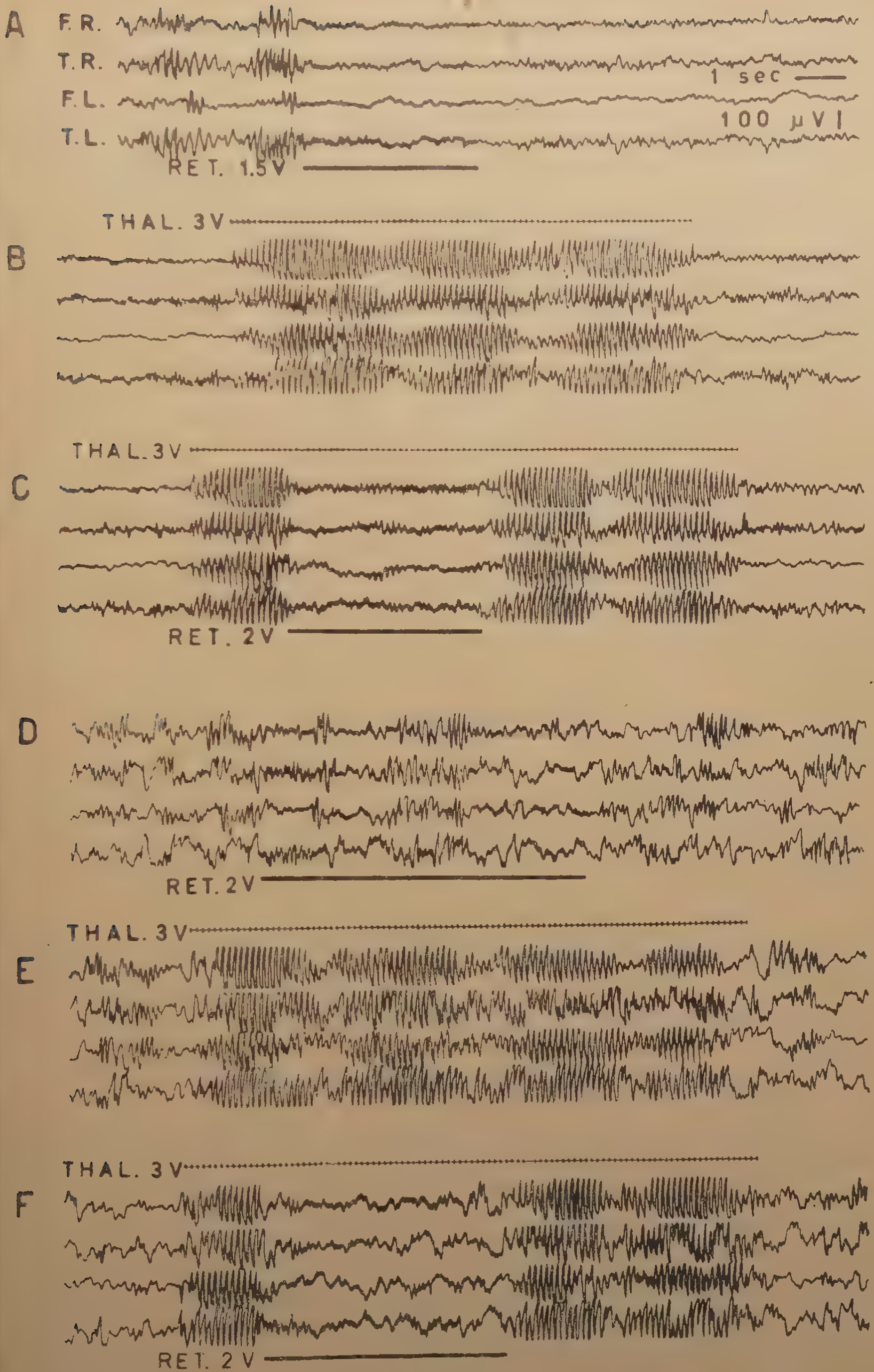
A
F.R.
T.R.
F.L.
T.L.
1 sec
100 μV
RET. 1.5V
THAL. 3V
B
THAL. 3V
C
RET. 2V
D
RET. 2V
THAL. 3V
E
THAL. 3V
F
RET. 2V

Fig. 3.

thalamic nuclei and in the cerebral cortex (20; Fig. 1: *A*, 4 and 5; Fig. 3: *C*). After injecting atropine in the usual doses capable of suppressing the activating reticular effect on the EEG (Fig. 1: *B*, 2; Fig. 3: *D*), we established that there was no modification in the reticular effects on the electrical activity induced by thalamic stimulation: the recruiting responses were still inhibited by reticular stimulation at intensities equal to the lowest effective intensity before the administration of the drug (Fig. 1: *B*, 4 and 5; Fig. 3: *F*).

Discussion

Before proceeding to discuss the possible mode of action of atropine on the reticular and thalamic mechanisms which modify EEG activity, it seems to us appropriate to summarise briefly the principal relevant results which have arisen either from earlier investigations by other workers or from our own researches reported above.

a) Atropine has no effect on the responses of the brain stem reticular substance to single electrical shocks applied to peripheral nerves or to sudden natural stimulations of brief duration (section 2 of our results, confirming the observation of Longo and Silvestrini (19) with scopolamine).

b) Atropine has no effect on the response of the rostral (mesencephalic) part of the reticular substance to stimulation with single electric shocks of the caudal (bulbo-pontine) part of the reticular substance itself (section 3 of our results).

c) Atropine abolishes the EEG arousal reaction produced by threshold electrical stimulation of the brain stem reticular substance (21, 17, 18, 6, 7; section 1 of our results). Supraliminal reticular stimulation can, however, produce a partial arousal reaction, consisting of a disappearance of the spindles (17, 18; section 1 of our results).

d) Atropine abolishes the EEG arousal reaction produced by high-frequency electrical stimulation of the diffusely-projecting thalamic system (17, 18; section 1 of our results).

e) Atropine has no effect on the thalamo-cortical (17, 18; section 1 of our results) or on the thalamo-thalamic recruiting responses (section 1 of our results).

f) Atropine has no effect on the inhibition, produced by high-frequency stimulation of the reticular substance, of the thalamo-cortical and thalamo-thalamic recruiting responses (section 4 of our results).

The findings referred to in *a*), *b*) and *f*) show respectively that atropine does not modify the normal conduction of impulses in the specific sensory pathways to the reticular substance, the conduction in the ascending intra-reticular pathways, or the reticulo-thalamic conduction. These points seem to us to provide a convincing direct proof that the reticular structures of the brain stem are not seriously depressed by doses of atropine which produce the typical synchronizing effect on the EEG. Our conclusions agree, then, on this point, with those of Bradley, Elkes and Key (5, 7, 2). Our findings also lead us to differ from the suggestion of Rinaldi and Himwich (21, 22), in that we do not consider that there are strong enough grounds for the hypothesis of the cholinergic nature of the ascending reticular system.

The situation is less clear with regard to the action of atropine on the diffusely-projecting thalamic system. Indeed we are faced with some apparently contradictory findings. On the one hand, atropine abolishes the arousal reaction which normally follows high-frequency stimulation of the aspecific thalamic nuclei (cf. *d*); on the other hand, the same doses of the drug do not in any way depress the recruiting responses produced by low-frequency stimulation of those same thalamic nuclei (cf. *e*), nor do they even alter the sensitivity of this recruiting activity to high-frequency stimulation of the reticular substance (cf. *f*). The first finding might lead one to suppose that the thalamic neurones were depressed, while the other findings would seem to indicate that the functional state of these structures was normal.

The simplest interpretation of this apparent contradiction between the results would be to suppose that the effect of atropine is to lengthen the phase of functional depression of the thalamic neurones which follows each electrical stimulus. In consequence, stimulations at low frequency, such as produce recruitment, would still be effective while stimulations at higher frequency, such as produce the arousal reaction, would cease to have any effect. The fact that the stimulation of the reticular substance at 300/sec suppresses the recruiting thalamic activity cannot, however, be easily reconciled with a lengthening of the refractory period of the thalamic neurones. It seems to us, rather, that this effect shows that the thalamic neurones have their normal sensitivity to impulses, presumably at high frequency, which reach them from the brain stem structures. This interpretative hypothesis could, however, be checked experimentally by studying the effects on behaviour of high-frequency stimulation of the aspecific thalamus before and after the administration of atropine.

A second possible interpretation would assign an extra-thalamic site, cortical or subcortical, to the effect of atropine. In the atropinized animal both low-frequency and high-frequency stimulation would excite the thalamic neurones in the normal way; these would as a result send on their synchronizing and activating impulses respectively to cortical and subcortical structures. Atropine would act on these structures in such a way, however, as to make them sensitive only to the recruiting impulses.

A third explanation could be, as already put forward by Longo (17, 18), that there are two aspecific thalamo-cortical systems topographically mixed, but functionally different or at least differentiable on the basis of the difference in their sensitivity to atropine. The one system, sensitive to the drug, would have an activating function; the other system, not sensitive to the drug, would have the opposite effect, recruiting or synchronizing. Evidence has been brought forward by King (16), and confirmed by Domino (12), for the possibility that the recruiting function of the thalamus is selectively affected by pharmacological agents which have no effect on the activating functions, and *vice versa*, but it seems to us difficult to prove that this selectivity of action necessarily involves the existence of two thalamo-cortical systems. Investigations carried out by recording the slow potentials of the cerebral cortex also lead one to the conclusion that the responses to stimulation at low frequencies (recruitment) and at high frequencies (arousal reaction) are the expression of two different functional states of one single system (9).

Whatever may be the correct interpretation of the mode of action of atropine at thalamic level, we are of the opinion that the results of our experiments lead to the following conclusions.

The abolition or the extreme depression of the arousal reaction produced by atropine must be due to a block of the influence of the ascending activating system. Insofar as the drug does not depress the excitability of the ascending reticular system and does not prevent the reticular impulses from acting normally on the recruiting responses of the thalamus, the site of this block would be *suprareticular*. Our experiments with electrical stimulation naturally do not allow us to say whether the tonic activity of the ascending reticular system is also normal after the administration of atropine, but the fact that the behaviour of the animal is not affected by the drug provides at least an indication (though not, of course, a decisive proof) in favour of the hypothesis that this tonic activity also is not seriously affected. If this thesis were accepted, we should be forced to concede that the EEG effect of atropine is not validly explained in terms of an inactivation of the ascending reticular system with consequent release of thalamic synchronizing mechanisms. It follows, therefore, that the explanation of the origin of the cerebral electrical activity produced by atropine is different from that commonly accepted for the EEG sleep produced by barbiturates or by transection of the midbrain (*cerveau isolé*). The hypothesis has been made (11; see however 10) that the spindles occurring during EEG sleep are due to thalamo-cortical volleys arising in the recruiting nuclei of the thalamus. If this explanation is accepted, a corollary would be that the pacemaker responsible for the EEG synchronization produced by atropine is not to be found, or at least is not to be found only, in the thalamic recruiting nuclei. Perhaps the thalamic recruiting system might be responsible for the spindles, insofar as the spindles are the only component of the cortical electrical rhythms induced by atropine which are still sensitive, although not always so, to reticular stimulation. The principal component of the atropine-induced synchronization, the slow waves, would then be due to the activity of extra-thalamic structures, subcortical or cortical, completely cut off from any ascending activating influence. This leaves open the problem of the identification of these structures and of the cause of their insensitivity to the action of the activating impulses.

Summary

1. A study has been made on the action of atropine on the cerebral electrical activity of the cat (curarized preparation, *encéphale isolé*, *encéphale isolé* submitted to trigeminal deafferentation, animals with partial destruction of the midbrain tegmentum).

2. It has been observed that atropine: *a*) produces sleep-like EEG patterns, *b*) inhibits the arousal reaction elicited by high-frequency electrical stimulation of the brain stem reticular substance and of the aspecific thalamic nuclei, *c*) has no influence on the

recruiting responses produced by low-frequency stimulation of the same aspecific thalamic nuclei, *d*) has no effect on the responses evoked in the reticular formation by single sensory stimuli, *e*) does not affect the responses evoked in the rostral midbrain reticular substance by single electrical shocks to the lower brain stem reticular structures, *f*) does not modify the inhibitory effect produced by high-frequency electrical stimulation of the reticular substance on the thalamically induced recruiting activity.

3. It is concluded that atropine does not depress the excitability of the ascending reticular system. The inhibition of the EEG arousal reaction is due to a supraretıcular, and perhaps suprathalamic, action of the drug. It seems that the origin of the sleep-like cerebral electrical activity produced by atropine is different from that of barbiturate narcosis and of the *cerveau isolé* preparation.

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INHIBITORY CONTROL OF SINOCAROTID PRESSOCEPTIVE AFFERENTS ON HYPOTHALAMIC AUTONOMIC ACTIVITY AND SHAM RAGE BEHAVIOR[1]

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It has recently been observed that electrocortical activity is influenced by stimulation of sinocarotid afferents, increased intrasinusal pressure resulting in EEG synchronization (6, 11). Since these EEG patterns are usually referred to a decreased activity of the ascending reticular system (see 34), the electrocortical effects of pressoceptive stimulation also have tentatively been considered to depend on a reflex inhibition of ascending reticular neurons (6, 11).

The experimental evidence that the sphere of influence of the pressoceptive sinocarotid afferents is not limited to the medullary vasomotor and respiratory centers suggested to us that it might spread also to other neural structures in some way related to the ascending reticular system. Among these structures we have considered the posterior hypothalamus, whose relationship to the reticular formation is so close that it is often conceived as the rostral portion of the latter. Indeed, arousal can be induced as easily by hypothalamic as by reticular stimulation (28), and lesions in the posterior hypothalamus and the midbrain tegmentum are both capable of precipitating behavioral and electroencephalographic patterns of sleep (21, 22). Since there is evidence suggesting that also those hypothalamic functions which are concerned with visceral

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regulation and emotional responses may be under the tonic control of ascending reticular impulses (7, 38), we have selected to study whether these neural structures are subject to the restraining influence of pressoceptive sinocarotid reflexes as well. Recently, the reverse relationship, *i. e.*, an hypothalamic influence on sinocarotid reflexes has been suggested by Gellhorn (16).

In shaping our experimental program, we have thought that any hypothetical influence on autonomic and somatic diencephalic activity might be better emphasized using a preparation displaying a high level of such hypothalamic activity. In the same way that the study of postural mechanisms has widely profited by the exaggerated postural reactions of the decerebrate animal, we have thought it likely that our study might take advantage of the strong background of hypothalamic excitability existing in the acute decorticate animal. Indeed, it is hardly possible to observe a more intense autonomic activity and excitability than in this preparation, whose "sham rage" behavior is classically considered as dependent upon central mechanisms located in the caudal hypothalamus (1).

Using this preparation, we have been able to demonstrate that *the diencephalic centers responsible for sham rage are under a tonic inhibitory influence from the sinocarotid, and probably aortic, pressoceptors.* Indeed a transient interruption of pressoceptor firing was regularly followed by a patterned outburst of hypothalamic somatic and autonomic activity, and, conversely, pressoceptive stimulation appeared to be capable of blocking or at least reducing frequency and intensity of the spontaneously occurring fits of sham rage.

METHODS

Our observations were performed in 65 cats, in acute conditions. Under ether anesthesia, an adequate opening was made in the left aspect of the skull, and a spatula was inserted into the lateral surface of the left hemisphere, approximately at the level of the *sulcus posterior*, and moved laterally up to the opposite cortex and then ventralward to reach the basal aspect of the brain at the prechiasmatic region. With this surgical approach, hemorrhage was in most cases negligible, and it was usual to obtain very active and long lasting preparations, with mean arterial pressures, as recorded from a cannulated femoral artery, averaging more than 100 mm Hg.

Previously to brain transection, both carotid sinuses were prepared according to the blind-sac technique by carefully tying all vascular branches arising from them, i. e. the external carotid, internal carotid, occipital and ascending pharyngeal arteries. In a number of preparations the external carotid artery was tied distally to the origin of the lingual artery, to permit cannulation of the proximal end of this vessel for introduction of lobeline and lycopodium into the blind-sac. In both cases the lingual circulation was interrupted and no leakage was permitted through this route. Two types of experiments were performed. In a larger series of animals, sinocarotid pressoceptor discharges were transitorily interrupted by decreasing intrasinusal pressure, which was simply obtained by clamping both common carotids. In a smaller group of animals the reverse experiment was performed: pressoceptors were stimulated by increasing intrasinusal pressure. For this purpose one or both common carotid arteries were ligated at the lower cervical level, cannulated and connected to a reservoir filled with warm saline; pressure inside the reservoir could be changed by means of a rubber pump and read on a mercury manometer. If one blind-sac only was prepared, the controlateral carotid sinus was denervated.

When selective inactivation of the chemoceptive sensitivity was required, this was effected bilaterally by injecting intrasinusally a small amount of a lycopodium suspension through the central stump of the lingual artery, conveniently cannulated (18). Chemoceptive sensitivity was tested previously and following embolization of the carotid bodies by recording the responses to small amounts (10 μg) of lobeline injected intrasinusally.

The behavior of the acute decorticate animal during inactivation or stimulation of the sinocarotid pressoceptors was carefully observed, and some of the autonomic and somatic phenomena were selected to be photographically recorded on a suitable multichannel apparatus (Battaglia Rangoni, Multistylus Special). Arterial pressure was measured from a cannulated femoral artery by means of a Sanborn capacitance manometer, and respiration was transduced by a crystal capsule connected to the side arm of the tracheal cannula. Graphic evidence of the somatic discharges paralleling autonomic activity in sham rage outbursts was obtained by leading the electrical activity of one or more muscles of the forelimbs, adequately amplified by Grass P-5 AC preamplifiers.

At the end of each experiment the level of brain transection was checked autoptically and in a few animals the brain was kept for subsequent histological examination.

Results

I. – *Effects of decreasing intrasinusal pressure*

1. *Carotid occlusion reflex in the acute decorticate cat.* — If the bilateral carotid occlusion was timed to occur during a period when the preparation was quiet, after a few seconds latency arterial pressure began to increase, breathing augmented in amplitude, pupils dilated, nictitating membranes became retracted, and soon phasic clawing movements, struggling, lashing of the tail and the characteristic facial expression of rage appeared. No such effect was ever obtained when the same neck region was touched without actual clamping of both common carotids. There is no doubt therefore that carotid occlusion elicited outbursts of activity which were thoroughly identical with those induced in the same preparation by any kind of peripheral stimulation, e. g. handling of a limb or twisting of the pinna (Fig. 1). In the most active animals, fits of

sham rage succeeded each other while the common carotids were clamped, gradually fading away.

Obviously, nobody can argue that in our experiments carotid occlusion might evoke hypothalamic discharges by affecting diencephalic neurons by transient local ischemia, since our experimental conditions were such as to have the whole brain continuously supplied by the vertebral circulation only, through the basilar circuit and the anastomotic branches of the circle of Willis. Clamping both common carotids could effect only the sinus region below the ligatures (blind-sac), but it was undoubtedly without any direct influence on brain circulation.

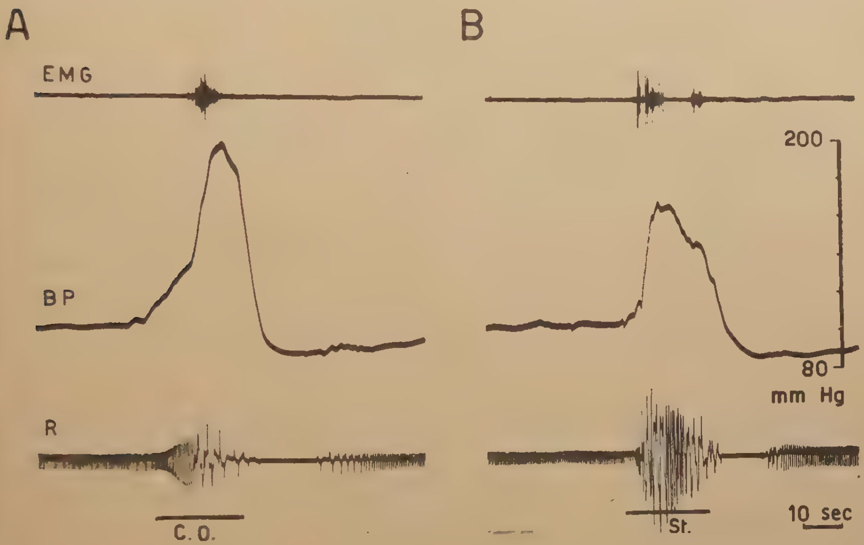

Fig. 1. – *Identity of sham rage patterns induced upon carotid occlusion and nociceptive stimulation in the vagotomized decorticate cat.*

In this and in the following Figs., EMG: electromyogram of triceps brachii of a forelimb; BP: blood pressure as recorded from a femoral artery; *R*: respiration.

A: effect of carotid occlusion (C. O.); *B*: effect of twisting a pinna(St).

It might be contended, however, that the increase in systemic arterial pressure consequent upon carotid occlusion may be capable of exciting diencephalic centers, e. g., by augmenting cerebral blood flow. Likewise, and more reasonably, reflex secretion of adrenaline and noradrenaline might in turn be responsible for the evoked hypothalamic activity. These objections have been ruled out in control experiments, performed following transection of the spinal cord at T 1. Although this procedure completely prevented any central nervous regulation of blood pressure or adrenomedullary secretion, sham rage outbursts could as easily be induced by carotid occlusion, provided that arterial pressure were maintained at reasonable levels (90-100 mm Hg) by a continuous intravenous infusion of minute amounts of noradrenaline (Fig. 2). Also when the spinal cord was transected at higher cervical levels, the facial components of sham rage could still be evoked. As this preparation has to be artificially ventilated, the possibility is ruled out that sham rage may be induced indirectly by the reflex hyperpnea, the consequent hypocapnia resulting in cerebral vasoconstriction.

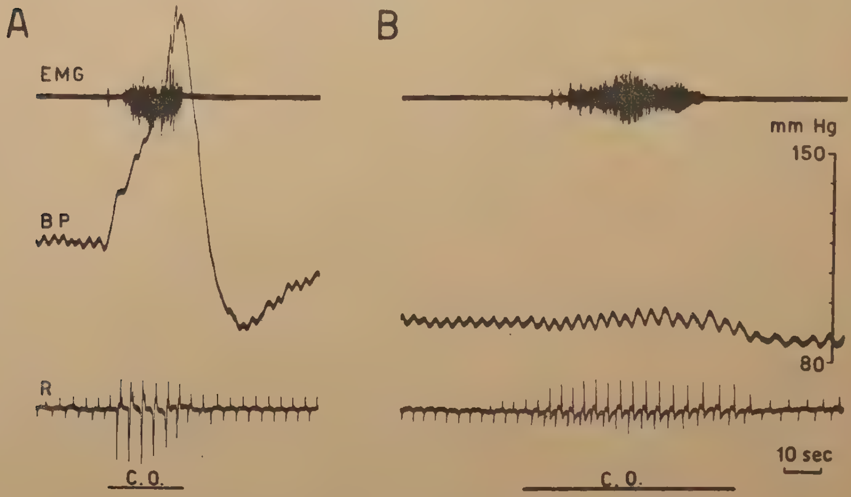

Fig. 2. – *Sham rage outbursts induced upon carotid occlusion before and after spinal cord transection.*

A: effect of carotid occlusion (C. O.) in the vagotomized decorticate animal. *B*: effect of carotid occlusion (C.O.) following section of the spinal cord at T 1. Note persistence of respiratory and motor patterns of rage, in spite of abolition of blood pressure changes.

Summing up, in acute decorticate cats carotid occlusion below a carotid sinus blind-sac induced outbursts of sham rage, which were independent of interference with brain circulation, of systemic pressure changes and of adrenomedullary secretion. It is therefore suggested that they depend on a reflex mechanism.

2. *Role of sinocarotid pressoceptive afferents.* — The definite demonstration that sham rage outbursts consequent upon carotid occlusion are related to sinocarotid reflex mechanisms is seen in Figure 3. Following denervation of both sinuses, the clamping of the common carotid arteries became completely ineffective, while sham rage fits could still be evoked be extero- or nociceptive stimuli (Fig. 3 B).

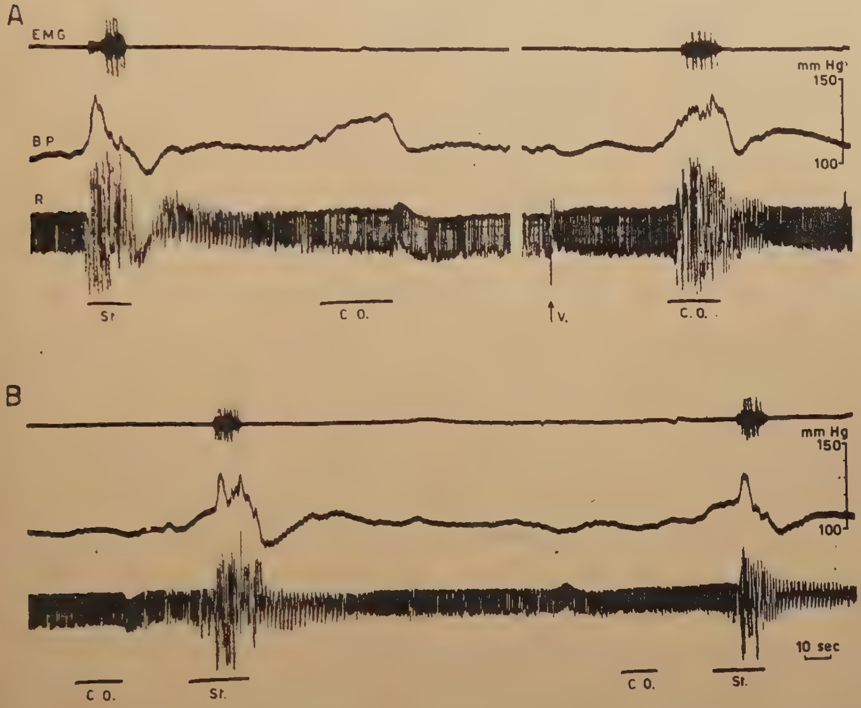

Fig. 3. – *Effects of vagotomy and subsequent carotid sinus denervation upon carotid occlusion response in the decorticate animal.*

A: decorticate cat; Hering's nerves and vagi bilaterally intact. Sham rage outbursts upon twisting of the pinna (St), but only blood pressure increase and hyperpnea without rage behavior upon carotid occlusion (C.O.). Following bilateral vagotomy (V), a sham rage outburst is evoked by carotid occlusion (see text p. 317).

B: following bilateral section of Hering's nerves (between *A* and *B*). Note that nociceptive stimulation (St) is still effective, while carotid occlusion (C.O.) yields no results.

If it can now be conceded that the outbursts of hypothalamic activity evoked by carotid occlusion are dependent on the sinocarotid reflex path, it cannot be concluded as yet that the observed

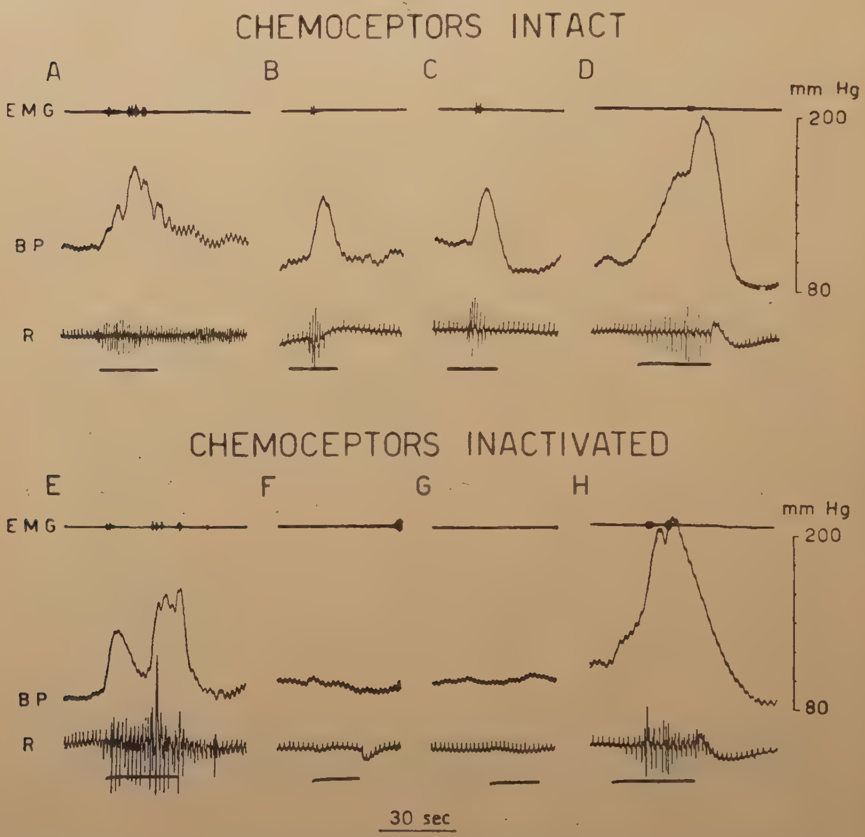

Fig. 4. – *Persistance of sham rage outbursts induced upon carotid occlusion following bilateral chemoceptive inactivation.*

Decorticate cat, bilaterally vagotomized. Compare effects of twisting a pinna (*A*, *E*), lobeline injection into the right (*B*, *F*) and left (*C*, *G*) carotid blind-sacs, and carotid occlusion (*D*, *H*) before (*A*, *B*, *C*, *D*) and following (*E*, *F*, *G*, *H*) bilateral inactivation of carotid body chemoceptors by lycopodium embolization. Note persistence of sham rage outbursts upon carotid occlusion (*H*) when local lobeline injection is thoroughly ineffective (*F*, *G*), owing to chemoceptive inactivation.

phenomenon really results from suppression of pressoceptive impulses. It might be contended that it depends on anoxic excitation of chemoceptors, since carotid occlusion does not only affect pressoceptive activity by decreasing the pulse pressure inside the sinus, but it may also excite the chemoceptors by stopping or slowing the blood flow through the carotid body (14, 15, 20). Indeed, we have demonstrated (5) in the decorticate animal that chemoceptive stimulation, either by intracarotid injection of lobeline or by systemic hypoxia, results in outburts of sham rage identical to those occurring upon carotid occlusion.

To assess the role of pressoceptive afferents in the phenomenon we were studying, we have resorted to selective inactivation of chemoceptive sensitivity, taking advantage of a technique described by Heymans and Bouckaert (18). When all arterial branches arising from the carotid sinus are tied, a lycopodium suspension introduced into the common carotid reaches the small arterial vessels leaving the initial part of the occipital or ascending pharyngeal arteries which supply the carotid body. Hence lycopodium introduction results in embolization and consequent inactivation of the chemoceptors, without disturbing the excitability of the neighboring pressoceptors. The effectiveness of chemoceptive inactivation was always tested by comparing the responses to intracarotid injection of lobeline (10 μg) preceding and following the embolizing procedure. As is shown in Fig. 4, injection of lobeline into the carotid circulation, which was so effective previously (*B*, *C*), appeared devoid of any action following bilateral embolization of the carotid bodies (*F*, *G*). In spite of the clear-cut inactivation of the chemoceptors, but with the pressoceptors remaining intact, carotid occlusion was still capable of evoking dramatic fits of sham rage (*H*).

Therefore, it appears safe to conclude that sham rage outbursts following decreased intrasinusal pressure are due to release from reflex inhibition arising from carotid sinus pressoceptors.

3. *Tonic nature of the inhibitory pressoceptive influence. Role of aortic afferents.* — The fact that transient interruption of pressoceptive activity results in outbursts of sham rage in the decorticate animal suggests in itself that hypothalamic centers are under a tonic inhibitory influence maintained by the continuous flow of pressoceptive impulses along the sinocarotid nerves. An attempt to assess this tonic inhibitory influence in more quantitative terms, by measuring thresholds for rage fits induced by electrical stimulation of the saphenous nerve, before and after prolonged carotid occlusion, was unsuccessful, since control thresholds varied considerably from moment to moment.

More definite evidence was occasionally obtained from rather inactive preparations, whose Hering's nerves were bilaterally severed at the end of the experiment. The results obtained from one such preparation are shown in Figure 5. Before pressoceptive deafferentation carotid occlusion was capable of evoking a moderate, although clear-cut, outburst of sham rage, but only hyperpnea without signs of rage behavior could be induced by twisting the pinna; no "spontaneous" outburst was present (*A*). When both Hering's nerves were severed, carotid occlusion became ineffective, but light, recurring fits of sham rage appeared anew in absence of any intentional extero- or nociceptive stimulation (*B*), as if the hypothalamus released from a tonic reflex inhibitory control, had become capable of discharging "spontaneously".

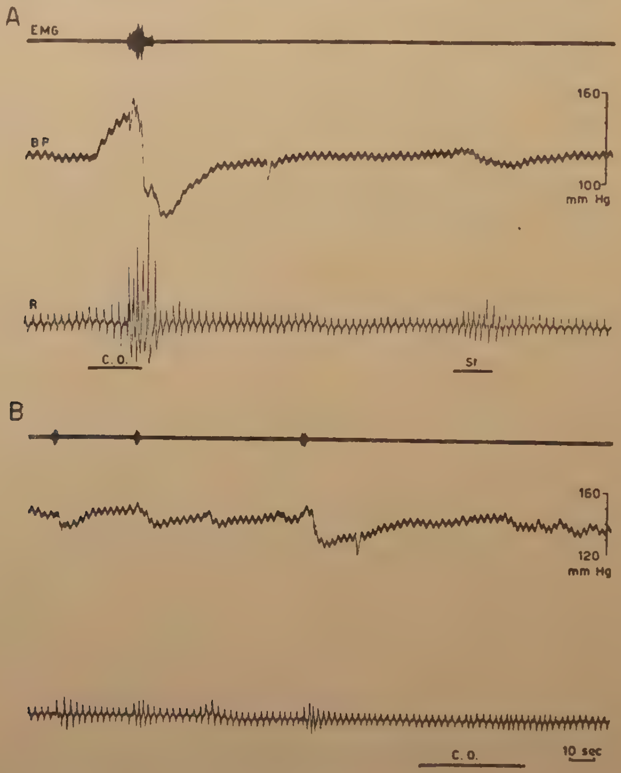

Fig. 5. – *Appearance of spontaneous outbursts of sham rage following pressoceptive denervation.*

Decorticate and vagotomized cat. *A*: sham rage fit upon carotid occlusion (C.O.); slight effects of nociceptive stimulation (St.). *B*: following bilateral section of Hering's nerves, carotid occlusion (C.O.) is ineffective, but mild "spontaneous" outbursts of rage activity, which were absent previously, are now evident (see EMG, left).

That the aortic pressoceptors, or at least vagal afferents, might contribute to this inhibitory influence was definitely suggested by the frequent observation that carotid occlusion resulted in outbursts of sham rage only after both vagi were cut. Fig. 3, *A*, for instance, shows a carotid occlusion reflex which was associated with further hypertension and hyperpnea and with struggling movements only after bilateral severance of the cervical vagi. Moreover, it was occasionally observed that rage fits evoked upon extero- or nociceptive stimulation were facilitated following bilateral vagotomy, an observation which is illustrated in Fig. 6. The frequent appearance of an outburst of rage upon cutting a vagus nerve (see Fig. 6) might well be suggested to depend on a release phenomenon, but it is likely to result from co-stimulation of vagal excitatory fibers as well.

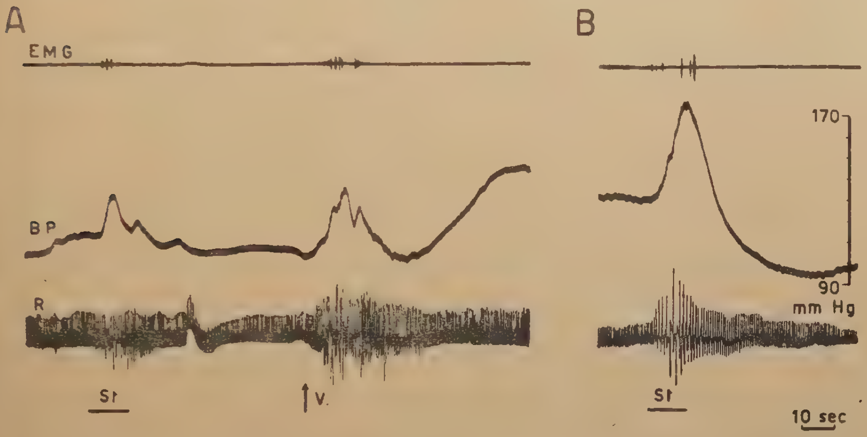

Fig. 6. – *Potentiation of sham rage activity following bilateral vagotomy.*
Decorticate cat. Both vagi intact. Sham rage activity evoked (*A*) by twisting the pinna (St) is exaggerated (*B*) after bilateral vagotomy (V), which is immediately followed by an outburst. Between *A* and *B*, 2 min.

Summing up, both sinocarotid and aortic pressoceptors exert a tonic inhibitory action on sham rage behavior. Pressoceptive deafferentation results, at least in some preparations, in an increase of somatic and autonomic outbursts, either spontaneous or evoked by exteroceptive stimulation.

4. *Suprasegmental contributions to the carotid occlusion reflex.* — It is well known from the classical experiments of Bard (1) that the caudal hypothalamus is indispensable for the appearance of

spontaneous emotional behavior or for evoking rage outbursts upon extero- or nociceptive stimulation. It is reasonable to expect that these same structures should remain functionally intact in order that the patterned responses of rage may be induced by carotid occlusion. More direct evidence pointing in this direction is presented here (see Fig. 7). In a few decorticate preparations, the central end of the lingual artery was cannulated with a fine polythene tubing, while all the vessels arising from the carotid sinus were left unclamped in order not to interfere with the carotid circulation to the brain. It has to be emphasized that these were the only animals in our experimental series in which a carotid sinus blind-sac could not be prepared. While transient carotid occlusion would elicit a rage fit in the decorticate animal (Fig. 7, *A*), introduction of a minute amount (0.8 mg) of a short acting barbiturate, Thiopental sodium, into the uninterrupted carotid circulation, through a cannulated lingual artery, prevented further appearance of rage outbursts either upon carotid occlusion or any kind of peripheral stimulation. Clamping of the common carotids still induced a moderate increase of arterial pressure and respiration, but no mydriasis or nictitating membrane retraction, nor any movement whatsoever: the patterned

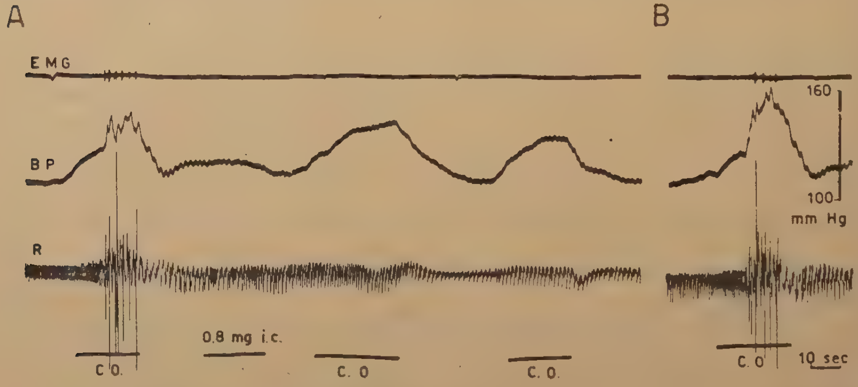

Fig. 7. – *Effects of intracarotid introduction of Thiopental upon responses voked by carotid occlusion.*

Decorticate, vagotomized cat. Right lingual artery cannulated. Outflow from carotid arteries otherwise free. *A*: effects of carotid occlusion (C.O.) before and after introduction of 0.8 mg Thiopental sodium into the carotid circulation through the right lingual artery. Note disappearance of somatic reactions (EMG), and reduction of respiratory responses. *B*: two minutes following Thiopental introduction, carotid occlusion is again capable of evoking outbursts of sham rage.

response of rage was completely lost. It returned only one or two minutes later, when the effects of the anesthetic drug were dissipated (Fig. 7, *B*).

A similar, but persistent, abolition of sham rage outbursts either upon carotid occlusion or nociceptive stimulation was observed when the decorticate cat was subsequently decerebrated by electrolytic coagulation at the posterior mesencephalic level. After introduction of Thiopental (0.8 - 1 mg) into the carotid circulation the respiratory and vasomotor responses to carotid occlusion were unmodified. These results clearly confirm the previous demonstration (23, 24) that small amounts of Thiopental introduced through the lingual artery are distributed only to those parts of the brain which are supplied by the carotid circulation, i. e. midbrain, diencephalon and forebrain, provided that blood flow through the carotid route is not interrupted during the injection.

Summing up, our results point more directly to the importance played by upper brain mechanisms located above the pontobulbar region in determining sham rage outbursts upon carotid occlusion. When these mechanisms are inactivated, either by drugs or by direct injury, the classical brain stem skeleton of the pressoceptive reflexes becomes apparent. Persistent activity in upper brain structures, probably diencephalic, is responsible for greater hypertension and hyperpnea, and for superimposed mydriasis, struggling movements and the whole patterned response of rage.

II. – *Effects of increased intrasinusal pressure*

While the aforementioned experiments of carotid occlusion seem sufficient to establish that an inhibitory influence is exerted by sinocarotid pressoceptors on brain centers responsible for rage behavior, and that such an influence is tonic in nature, further research appeared necessary to gain more information on the extent and the effectiveness of such an inhibitory action. It was therefore decided to test increased pressure in the carotid blind-sac on spontaneous and evoked outbursts of sham rage.

In this group of experiments it was found exceedingly difficult to block or even to restrain the hypothalamic fits reflexly induced by extero- or nociceptive stimuli. Active inhibition by stimulated pressoceptors could be revealed in favorable preparations only, displaying regularly and frequently recurring " spontaneous " outbursts of sham rage. Playing upon such a background of rhythmic hypothalamic discharges, it could be demonstrated that abrupt raising of intrasinusal pressure from 0 to 200 mm Hg was capable of blocking or at least decreasing in frequency and strength the somatic and autonomic fits (Fig. 8, *A*). On releasing the intrasinusal pressure, a rebound outburst was constantly obvious. It is interesting that, in the same preparations, when the rhythmic rage behavior was reinforced by periodic tapping of a limb or scratching of the neck region, comparable increase of intrasinusal pressure was insufficient to keep in abeyance the exteroceptively evoked or reinforced hypothalamic discharges (Fig. 8, *B*). Although the vasomotor

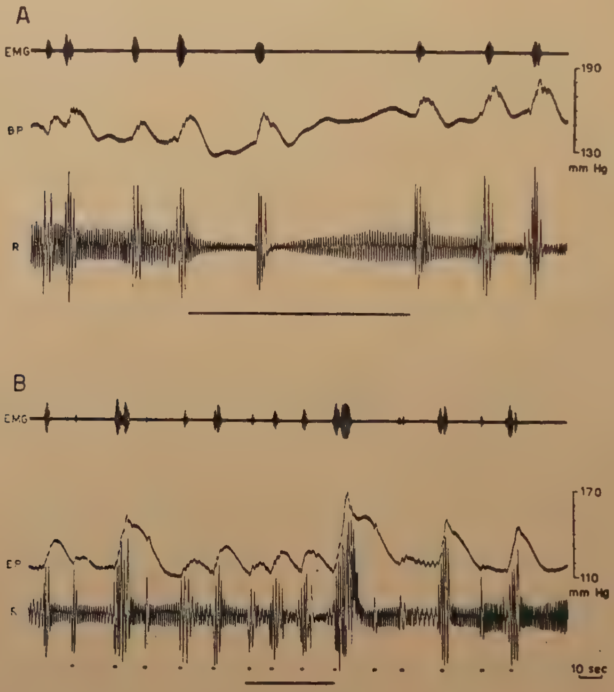

Fig. 8. – *Effects of increased intrasinusal pressure upon " spontaneous " and evoked outbursts of sham rage.*

Decorticate, vagotomized cat. Left carotid sinus denervated; right carotid sinus blind-sac; chemoceptors inactivated (lycopodium embolization). *A*: partial inhibition of spontaneously occuring outbursts of sham rage during increase of pressure inside the right carotid sinus from 0 to 200 mm Hg (continuous line). *B*: no definite effect of comparable pressoceptive stimulation (continuous line) on outbursts of sham rage driven by rhythmic scratching of the neck (dots).

and, to some extent, the respiratory components of the rage behavior were definitely decreased, probably because of the classical inhibitory effect at the medullary level, struggling movements and, on the whole, the patterned response did not appear to have undergone any important modification. Here again, however, cessation of pressoceptive stimulation was followed by a rebound increase, at least of the autonomic components of the rage behavior. It must be kept in mind, moreover, that the use of a steady pressure in the carotid sinuses might have somewhat reduced the effectiveness of pressoceptive stimulation, especially when this was protracted for several seconds (13, 19). This is suggested also by the slow rising of arterial pressure which is evident in Fig. 8, *A* during the later part of pressoceptive stimulation, but it appears unlikely, on the whole, that, at the very high intrasinusal pressure we have used, the steady character of the stimulus might have severely impaired the observation of more dramatic responses (13).

It can be concluded that, while pressoceptive discharges exert an effective inhibitory influence on the spontaneous rhythmic activity of the nervous centers which are responsible for rage behavior, this reflex inhibitory control is easily overwhelmed by co-stimulation of sensory afferents playing an excitatory role upon the same brain mechanisms.

DISCUSSION

Before commenting on the significance of carotid inhibition of rage behavior, it seems necessary to discuss the level wherein and the pathways whereby such restraining influence is exerted.

The classical opinion that bulbopontine centers are the target of the reflex pressoceptive control (see 19) might suggest that all components of the patterned response of rage are inhibited at the pontine and medullary level. We have no direct proof against this hypothesis; however, it seems hardly possible that an outburst of rage might be elicited during periods of apparent quiescence of the animal, simply by release of the bulbopontine autonomic and somatic centers. Moreover, when intracarotid injection of Thiopental or brain stem transections change the decorticate into a decerebrate preparation, circulatory and respiratory reactions are still evoked by pressoceptive inactivation, but no pupillary, ocular or somatic phenomena can then be observed. Finally, rage responses induced by carotid occlusion in the decorticate animal are indistinguishable from those spontaneously occurring or evoked by exteroceptive stimulation. Since it is conceded that the effects of the latter type of stimulation result from an excitation of the diencephalic structures responsible for rage behavior, one does not see why it should be amiss to conclude that when identical patterns of response are evoked by carotid occlusion, they result from a release and a consequent increase in activity of the same hypothalamic structures.

This conclusion does not necessarily imply that neurons of the caudal hypothalamus are actively and directly inhibited by pressoceptive firing. Since the posterior hypothalamus is supposed to be within the sphere of influence of the ascending reticular formation (7), and this is in some way inhibited by pressoceptive discharges (6), sinocarotid reflexes might influence hypothalamic centers simply by an indirect deactivation, i. e., by restraining the tonic reticular drive indispensable for maintaining or discharging hypothalamic activity.

A partially different explanation is suggested by the existence of an EEG synchronizing and possibly sleep inducing influence, which has been shown (3, 4, 2, 23, 9) to originate from the lower brain stem, i. e., from regions wherein sinocarotid afferents are known to terminate. Although the tonic activity of this synchronizing mechanism appears to be independent of pressoceptive impulses, so far at least as electrocortical patterns are concerned (9), it is only reasonable to suggest that pressoceptive impulses might utilize this pathway to exert their influence both on the cerebral cortex and on the hypothalamus. Whether such a deactivating mechanism of the lower brain stem should act upon the hypothalamus through direct pathways or indirectly, e. g. through an inhibition of the midbrain reticular formation, cannot as yet be decided.

While these problems remain open to further research, the present experiments point out that those hypothalamic centers which are considered as responsible for rage behavior are under a more complicated regulation than was previously supposed. To the well known "descending" inhibitory control coming from as yet ill defined regions of the forebrain, a new restraining influence "ascending" from peripheral receptors has now to be added. However, not to be tempted by misleading oversimplifications, the different strength of the two "inhibitory" mechanisms has to be stressed. Indeed, while following "decortication" sham rage outbursts can easily be evoked with pressoceptors intact, mere pressoceptive deafferentation is incapable of bringing forth hypothalamic overactivity in the intact animal. The restraining role of forebrain structures is obviously much more powerful than that played by the pressoceptors. Furthermore, as we have shown, the latter influence is effectively opposed by the reflex excitatory action of a wealth of peripheral afferents, which usually play an overwhelming role, at least in the decorticate animal. However, one should not be amazed to see how easily concomitant excitatory stimuli can conceal the reflex inhibitory influence. In commenting on the functional balance of the reticular formation, it has been suggested (37) that the tonic activity of the reticular structures is likely to be supported by a powerful excitatory drive, so that reflex inhibition may be brought to light only when firing from the periphery is not too strong and intensity of central excitation is not too high. Therefore, the posterior hypothalamus seems to share one more property with the reticular formation, or to be strictly dependent upon the level of activity of the reticular system itself. At any rate, it would be misleading to underestimate the inhibitory contribution of pressoceptive reflexes in setting or tuning the level of hypothalamic excitability. Such a contribution is far from being irrelevant, if its phasic suppression can bring the diencephalic activity of the decorticate animal out of apparent silence, and its permanent suppression seems capable of decreasing the threshold of diencephalic activation.

One more conclusion can be drawn from our experiments. An increasing number of observations have recently suggested that the sphere of influence of sinocarotid and aortic pressoceptors is not limited to the bulbopontine vasomotor and respiratory centers but includes brain stem mechanisms involved in the regulation of muscular tone (12, 32, 33, 35) and electrocortical activity (6, 29, 30, 27, 26). Our findings fully agree with the results of these previous investigations. However, all the preceding observations were concerned with regulation of isolated functions which by themselves cannot characterize any specific behavior, or were performed on preparations unsuitable for a behavioral study. Our results in the decorticate cat clearly point out that the pressoceptive influence may be exerted not only on peripheral centers of autonomic and somatic motor outflow, but also on higher nervous mechanisms which are involved in the control of complex patterned responses having a definite behavioral significance. Of course, we are not ready to state that carotid sinus reflexes have any impact on emotions also, since we are fully aware of the ancient dispute as to whether rage fits in the decorticate animal are associated with subjective emotion, or simply represent an exacerbation of the patterned motor activity whereon emotion eventually plays (1, 31, 25, 17, 36, 10, 8). At any rate, even if one holds to the latter viewpoint that hypothalamic reactions are really "sham" or "pseudoaffective", as they are classically named, it is equally important to see how impulses originating from vascular receptors spread their influence upon multifarious autonomic phenomena and somatic reactions as well, keeping in abeyance complex patterned responses which at least subserve consciousness and emotion.

A final point has to be made. The existence of a tonic reflex inhibition on hypothalamic activity suggests that the latter also is likely to be tonic in nature, at least in the decorticate animal, where simple removal of the steady pressoceptive influence can bring forth "spontaneouslike" outbursts of hypothalamic activity. The finding that such a background of central activity is finely modulated by peripheral receptors having a well known bearing on homeostasis of autonomic functions hints that the role played by the posterior hypothalamus and its related structures in the normal regulation of autonomic functions may be more important than previously supposed.

SUMMARY

1. In acute decorticate cats, carotid occlusion below a carotid sinus blind-sac induced outbursts of sham rage which were potentiated by bilateral cervical vagotomy, and abolished by section of Hering's nerves.

2. Sham rage outbursts followed carotid occlusion even when carotid body chemoceptors had been inactivated by embolization with a lycopodium suspension, a finding showing that this effect of carotid occlusion results from transient suppression of pressoceptive discharges, independently of chemoceptive co-excitation.

3. Upper brain mechanisms are indispensable for the appearance of sham rage patterns upon carotid occlusion, since the latter procedure failed to evoke any pseudoaffective reactions when midbrain and diencephalon were temporarily inactivated by injection of a minute dose of Thiopental into the external carotid circulation.

4. Pressoceptive stimulation by increasing intrasinusal pressure succeeded in blocking spontaneously occuring outbursts of sham rage, but appeared much less effective in influencing evoked pseudo-affective activity.

5. It is concluded that the central neural mechanisms responsible for sham rage behavior are also within the sphere of the inhibitory influence of sinocarotid pressoceptors.

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ANALYSES

Progress in Biophysics and Biophysical Chemistry. Vol. IX, J. A. V. Butler and B. Katz, Editors. New York and London, Pergamon Press, 1959, 390 pp., $ 17,50

The domain of " Biophysics and Biophysical Chemistry " is so large that this reviewer need not apologize for being on some terms of familiarity with the subject matter of only four of the seven chapters and with intimate knowledge of only two of these, by W. A. H. Rushton (Cambridge) on "Visual Pigments in Man and Animals and their Relation to Seeing, " and by J. A. B. Gray (London) on " Mechanical into Electrical Energy in Certain Mechanoreceptors. "

Two of the other 5 chapters are closely related topics: " The Kinetics of Reactions between Haemoglobin and Gases, " by Q. H. Gibson (Sheffield) and « Diffusion and Simultaneous Chemical Reaction Velocity in Haemoglobin Solutions and Red Cell Suspensions, " by F. J. W. Roughton (Cambridge). Gibson limits himself to a clear and general presentation of the problems and concepts that are in the forefront at the present time in the field. Roughton emphasizes in considerable detail a mathematical and quantitative treatment aiming: " (i) To summarize the work done on the development of theoretical solutions, both by analytical and by numerical approximation methods, for the uptake of oxygen, carbon monoxide and nitric oxide in flat layers and in spheres of haemoglobin solution, with or without bounding membranes. (ii) To discuss the application of this theoretical treatment to the various experimental observations of these processes.... "

As a student at the time, I followed eagerly the exciting new approaches of Hartridge and Roughton in the 1920's. After long estrangement, Gibson's conclusion (pp. 50-51) strikes some notes made familiar by specialization in entirely different work: " The kinetics of the reactions of haemoglobin with gases have now been under active investigation for some 30 years. At almost any time during that period it would have been possible for a reader not actively engaged in the field to conclude that final and definitive description of these reactions was at hand. In the event, the application of new technical methods, while confirming the accuracy of the experimental observations which had gone before, has revealed new difficulties and complexities even in apparently simple situations. It is perhaps fair to say that we now know enough about the kinetics of the reactions of gases with haemoglobin to deal with the physiological problems of oxygen transport in the circulation, and that further work is being undertaken in order to find out more about the reactions for their own sake. This is desirable because haemoglobin may well be taken as a model for the action of enzymes, and the complexities met with in working with it may prove a pointer to much greater complexities likely to appear when the study of enzyme kinetics can be carried to similar lengths. "

Self-doubt is healthy for every active branch of science, as is also the look forward to correlations with other aspects of biology. Indeed, the relation which Gibson looks to with enzyme kinetics may be generalized even more broadly. Biology is coming to grips, though as yet slowly, with the need to analyze in detail the behavior of organized systems of the cell, and particularly of the cell membranes. Signs are clearest in electrophysiology and in the study of secretion and uptake of specific substance, but studies on the processes of " induction ", in pharmacology, in bacterial genetics and biochemistry, and in experimental embryology also reflect the trend. We can all the more easily accustom ourselves to regard the phenomena and their interrelations with appropriate sophistication when we realize that so " simple " a system as haemoglobin also discloses baffling structural and dynamic complexity.

A familiar note, particularly to a neurophysiologist in these days of " electronic brains ", is an observation by D. A. MacDonald and G. M. Taylor (London) in their chapter on " The Hydrodynamics of the Arterial Circulation " (p. 109): " It is obvious that when dealing with a system of such peculiar and complex properties, many simplifying assumptions must be made to start a theoretical analysis; but past experience has shown that simplifications made explicitly as an analogy tend to be inherited by successive workers, until the abstractions of the analogue have been taken for reality. "

I cannot make any useful comment on the contents of this chapter, nor on the next, by A. Peterlin (Ljubljana) on " Determination of Molecular Dimension from Light Scattering Data " or the last, on " Gradient Centrifugation of Cell Particles: Theory and Applications ", by C. de Duve, J. Berthet, and H. Beaufay (Louvain). These two bracket the two chapters on sensory physiology to which I referred above.

Rushton presents, very elegantly, a unifying picture which is based largely on new experimental work from Cambridge, woven into a background of other data on biochemistry and physiology of visual processes. His analytical treatment leads to a clearly stated theoretical position. Unfortunately, there seems to have been considerable time lag between the preparation of the paper and its publication. This is an unfair handicap against an author in a field which is developing rapidly. Svaetichin's and Tomita's data on electrophysiology of fish retina are not discussed, nor is my subsequent theoretical analysis of the electrophysiology of photoreceptors in terms of the general hypothesis of electrically inexcitable electrogenic processes.

As a student of the late Selig Hecht's, I may be overly sensitive to what appears to me neglect, more so by others than by Rushton, of Hecht's contributions to the theory of color vision. Rushton cites Hecht's 1937 article in Physiological Reviews, but the section on " Visual Pigments in the Cones " (pp. 268-279) has only one mention of Hecht's work, reference to the latter's " celebrated equation " (p. 277). Hecht's pioneer attempt to correlate a very large body of psychophysical data in terms of a single theory, as detailed in articles in Murchison's Handbook, Ergebnisse der Physiologie, or the Howe Lectures, is not mentioned. Yet, Rushton's own data on color blinds to a considerable extent relate to Hecht's theory. The passage of time inevitably disclosed defects in the latter, some of which Hecht himself recognized before his untimely death. He would have welcomed with enthusiasm the remarkable work of Rushton and his collegues, and undoubtedly would have himself contributed to the new developments.

Gray states that (p. 288) " The problem to be discussed is the mechanism by which certain mechanoreceptors produce receptor potentials in response to mechanical changes. " In point of fact, this is one of the areas in which the electrophysiologist feels painfully ignorant. At present we have not even an inkling of the events which transpire when a stimulus modifies the molecular structure of an electrogenic membrane so that the membrane develops its transducer action. Most of us are agreed that this results in movements of ions which produce a change in membrane potential. However, as Gray correctly states in his brief discussion of " Theories of Mechanical Action " (pp. 316-318) " there is at present no positive evidence as to the nature of the process " that is involved in the transducer action.

Gray concentrates, quite usefully, on the properties of receptor potentials (pp. 295-302), on the nature of the ionic processes (pp. 311-316), and on what he terms " specialization of the terminals " (pp. 307-311), dilating (pp. 308-310) upon " absence of impulses " in these. Gray and his colleagues arrived independently at a view of generator potentials which is related to, but formulated less precisely than one that I developed in connection with the concept of electrically inexcitable electrogenesis. Evidence supporting the electrical inexcitability of receptor membranes is now available from work on visual sensory systems and on osmo- and mechanoreceptors, including the Pacinian corpuscle, but Gray ignores it. His treatment thereby becomes essentially phenomenological, lacking the generalizing insight which is made possible by the recognition of the fundamental difference between receptive membranes and the conductile, and of its consequences.

Theoretical papers dealing with this subject and available since 1957 are not cited. Indeed, except for some " intramural " papers there are remarkably few reference to publications since 1957 in Gray's and, as already noted, also in Rushton's papers. Perhaps the authors were required to close, sometime in 1957, manuscripts that are now published in a 1959 volume. In that case the editors and the publishers ought to indicate that fact. Authors in the various " Annual Reviews " state the date at which their surveys terminated. This is an admirable policy which ought to be more widely adopted.

H. Grundfest

H. K. Beecher. *Measurement of subjective responses: quantitative effects of drugs*. Oxford University Press, New York, 1959, xvi-494 pp., $ 12.75.

The problem of pain, perhaps more than any other aspect of biology, has consistently led to heated controversies in the scientific literature. The bitter and vituperative arguments that originated with Von Frey and Goldscheider have been carried on in milder form until the present time, and new controversies have arisen in the course of exploration of the pain problem. Despite a vast amount of work, few (if any) aspects of pain are agreed on by all the workers in the field: the adequate stimulus for pain has never satisfactorily defined; the peripheral fiber groups and the CNS pathways involved in transmission have not been unequivocally ascertained; and there is still controversy about the nature of the ultimate perceptual event.

There are so many facets to the problem of pain that only a few men have succeeded in achieving a command of the whole field. One of these is Henry K. Beecher, Professor of Anesthesia at Harvard Medical School. His primary interest is the measurement of pain, but he has explored such related responses as nausea, euphoria and anxiety as well. In a field notorious for generalizations based on one or two uncontrolled clinical observations, Beecher stands out for his comprehension of experimental techniques. Beecher has for some time now argued effectively for scientific controls and statistical analysis in the study of drug-effects on subjective states, and his new book, *Measurement of subjective responses*, represents the most forceful, complete exposition of his ideas to date.

The book is composed of two sections. The first deals primarily with pain and the problems encountered in the attempt to achieve controlled techniques for studying the effects of drugs on pathological pain states. The second section contains the results of a series of clinical investigations on subjective states: the measurement of " mental clouding, " the effects of psychomimetic drugs, sedatives and hypnotics, the induction of euphoria and dysphoria by various drugs, and the effects of drugs on nausea, anxiety, itching and hunger. The first section of the book, then, presents an approach to the problem of subjective states and provides the prototype for the experimental studies reported in the second section.

One of the major controversies in the field of pain at present concerns the methods of evaluating pharmacological agents for their effectiveness in relieving pain. Morphine has long been known to have a profound effect on pain — this is an established clinical fact. Yet to show that this is so experimentally has been an astonishingly difficult task. Beecher argues effectively against attempts to evaluate drugs when the source of pain is radically different from that which is to be treated by the drugs. In particular, he musters his forces against the widely-used radiant heat technique proposed by Wolff, Hardy and Goodell. Beecher prefaces his book by saying that « Many investigators seem grimly determined to establish — indeed, too often there does not seem to have been any question in their mind — that for a given stimulus there must be a given response; that is, for so much stimulation of pain endings, so much pain will be experienced.... This fundamental error has led to enormous waste.... Work presented here makes it clear that there is no simple relationship between stimulus and subjective response " (p. x.). Beecher then moves into the debate against Wolff, Hardy and Goodell armed with a scholarly knowledge of the literature (there are more than a thousand references), a splendid collection of experimental data from his own laboratory (at the Massachusetts General Hospital) and a capacity to express himself well in a clear and forthright manner.

The book, then, has two goals: first, to propose a method for the evaluation of pain and to demonstrate its effectiveness, and second, to attempt to clarify knowledge on pain mechanisms as a result of his investigations.

Pain, argues Beecher, is a function of a multitude of contributing factors (with the stimulus representing only one of these contributions), and a reliable and valid evaluation of drugs can only take place in the clinic where all of these factors are present. Afetr reviewing the great variety of techniques for studying pain (Chapter 2), Beecher goes on to point out the amazing inconsistency of results that have been recorded. One of the sources of great error in many of the techniques is the lack of adequate controls capable of showing that a given drug can effectively reduce pain beyond the effect of a placebo. Chapter 3, which deals with " placebos and placebo reactors ", makes fascinating reading. By using the " double-blind technique " and a sophisticated statistical analysis, Beecher presents convincing data to show that a large dose of morphine (15 mg/ 70 kg body weight) relieves 75% of patients suffering post-operative pain while a placebo such as saline is effective for about 35% of patients. That is, " of the average pain relief produced by a large dose of morphine in treating severe pain, nearly half must be attributed to a placebo " effect (p. 65).

This extraordinary power of placebos is bound to have a disastrous effect on experiments which fail to safeguard against it, and Beecher describes in detail the experimental design and statistical analytic methods essential for a valid and reliable outcome. In addition, he includes a valuable chapter on experimental design and statistical analysis prepared by Dr. Frederick Mosteller, Professor of Mathematical Statistics at Harvard. One chapter in the first section, which describes an experiment on the potentiating effects of a non-analgesic drug (papaverine) on the analgesic action of morphine and almost all of the second section illustrate convincingly the effectiveness of Beecher's techniques in obtaining meaningful data. Beecher also includes such essential information as the criteria for pain relief by using the scaling methods of psychophysics, the development of questionnaires, and useful ways of categorizing subjective states. Thus the first aim of the book, to elucidate a useful method for studying subjective responses, is met in a highly satisfactory way.

Beecher also successfully achieves his second goal: to throw light on the nature of pain mechanisms. In three chapters dealing with the pain threshold (Chapters 5, 7 and 8) he succeeds in demolishing the argument of Hardy, Wolff and Goodell that pain thresholds are constant among different people and in the same person at different times. The evidence is overwhelming that the pain threshold in man is not constant, and Beecher's findings point clearly to the cause: the powerful contribution to the total pain experience made by psychological factors such as attention, the meaning of the pain to the person, the situation in which it is received and so on. In chapter 9 he deals with these components in detail, and reports his now-famous observations on seriously wounded men during World War II: " Of the wounded soldiers about one-third .... wanted medication to relieve their pain and two-thirds did not. Of the civilians suffering from far less tissue trauma four-fifths wanted medication to relieve their pain and one-fifth did not. Thus the figures are reversed. While the details are discussed elsewhere, the important difference in the two groups seems to lie in their responses to the wounds. In the wounded soldier it was relief, thankfulness for his escape alive from the battlefield, even euphoria (his wound was a good thing); to the civilian his major surgery, even thought essential, was a depressing, calamitous event. The civilian group's pain was strikingly more frequent and more severe than that of the soldiers. These data state in numerical terms what is known to all thoughtful clinical observers: There is no simple, direct relationship between the wound *per se* and the pain experienced. The pain is in very large part determined by other factors, and of great importance here is the significance of the wound.... " (p. 165).

Those interested in pharmacology, psychology, and physiology will find this book helpful, not only for the ideas but also for the scholarly and skillful review of the relevant literature. Beecher has opened new roads into the study of pain perception and subjective states generally. His book is bound to have an important influence on our understanding of the nature of pain and of the effects of drugs on conscious experience.

R. MELZACK

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